

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 October 2005 (13.10.2005)

PCT

(10) International Publication Number
WO 2005/094420 A2

- (51) International Patent Classification: Not classified
- (21) International Application Number:
PCT/US2005/004954
- (22) International Filing Date: 17 February 2005 (17.02.2005)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/521,072 17 February 2004 (17.02.2004) US
- (71) Applicant (for all designated States except US): **UNIVERSITY OF SOUTH FLORIDA** [US/US]; 4202 East Fowler Avenue, FAO 126, Tampa, FL 33620-7900 (US).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **MOHAPATRA, Shyam, S.** [US/US]; 18510 Country Crest Place, Tampa, FL 33647 (US).
- (74) Agents: **LADWIG, Glenn, P.** et al.; Saliwanchik, Lloyd & Saliwanchik, PO Box 142950, Gainesville, FL 32614-2950 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MATERIALS AND METHODS FOR TREATMENT OF INFLAMMATORY AND CELL PROLIFERATION DISORDERS

(57) Abstract: The present invention pertains to methods for treatment of inflammatory and cell proliferation disorders, such as cancer, by administering an agent that reduces atrial natriuretic peptide receptor-A (NPR-A) activity. In one aspect, the invention concerns a method for treatment of inflammatory and cell proliferation disorders, such as cancer, by administration of an effective amount of natriuretic hormone peptide (NP), or a polynucleotide encoding NP and an operably-linked promoter sequence. In another aspect, the present invention includes a pharmaceutical composition comprising an agent that reduces the activity of atrial natriuretic peptide receptor-A (NPR-A), and an anti-cancer agent. In another aspect, the present invention further concerns a method for identifying an agent useful for treating an inflammatory or cell proliferation disorder, comprising determining whether the agent reduces the activity of atrial natriuretic peptide receptor-A (NPR-A).



WO 2005/094420 A2

DESCRIPTIONMATERIALS AND METHODS FOR TREATMENT OF INFLAMMATORY
AND CELL PROLIFERATION DISORDERS

5

Cross-Reference to Related Application

The present application claims the benefit of U.S. Provisional Application Serial No. 60/521,072, filed February 17, 2004, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, or drawings.

10

Background of the Invention

The vast majority of cancers of the lung, breast and colon are adenocarcinomas, which arise from pre-existing adenomatous polyps that develop in the normal colonic mucosa. This adenoma-carcinoma sequence is a well-characterized clinical and histopathologic series of events with which discrete molecular genetic alterations have been associated. Lung tumor development and metastasis are complex processes that include transformation, proliferation, resistance to apoptosis, neovascularization, and metastatic spread. A number of gene products have been identified that play critical roles in these processes. It has been suggested that the development of epithelial-derived tumors, the most common class of cancers, involves mutations of tumor suppressors and proto-oncogenes or epigenetic alterations of signaling pathways affecting cell proliferation and/or survival, which in turn may be caused by inflammation induced by infections and reactive oxygen species (ROS) (Ernst, P. *Aliment Pharmacol Ther.*, 1999, 13(1):13-18).

15

20

25

30

A group of four peptide hormones, originating from the 126-amino acid atrial natriuretic factor (ANF) prohormone, have become known for their vasodilator activity. These four peptide hormones, consisting of amino acids 1-30, 31-67, 79-98, and 99-126 of this prohormone, have been named long acting natriuretic peptide (LANP), vessel dilator (VD), kaliuretic peptide (KP), and atrial natriuretic peptide (ANP), respectively, for their most prominent effects (Angus R.M. *et al.*, *Clin Exp Allergy* 1994, 24:784-788). The ANP sequence, particularly the C-terminal portion, is highly conserved among species (Seidman *et al.*, *Science*, 1984, 226:1206-1209). ANP has been proposed to be

useful for treatment of various cardiovascular, respiratory, and renal diseases (Vesely, D.L. *Cardiovascular*, 2001, 51:647-658), but also causes inflammation. The family of natriuretic hormone peptides has been shown to have broad physiologic effects, including vasodilation and inhibition of aldosterone secretion and cardiovascular homeostasis.

5 As indicated above, ANF, the 126 amino acid prohormone, gives rise to four peptides: LANP (amino acids 1-30), VD (amino acids 31-67), KP (amino acids 79-98) and ANP (amino acids 99-126, also referred to herein as NP₉₉₋₁₂₆) (Angus R.M. *et al*, *Clin Exp Allergy*, 1994, 24:784-788). The ANP sequence particularly the C-terminal portion is highly conserved among species (Seidman *et al.*, *Science*, 1984, 226: 1206-1209). The
10 natriuretic peptide receptors (NPRs), NPR-A and NPR-B, are expressed in many different tissues of various organs systems, and are coupled to guanylyl cyclase. ANP and BNP are thought to signal primarily through NPR-A by increasing cGMP and activating cGMP-dependent protein kinase (PKG). NPR-A is the primary receptor for ANP while NPR-B seems to bind CNP most effectively. PKG activation in turn activates ion
15 transporters and transcription factors, which together affect cell growth and proliferation, apoptosis and inflammation. NPR-C is a clearance receptor for ANP removal, but also appears to signal phospholipase C activation and a decrease in adenylyl cyclase activity through a cGMP-independent pathway (Abbey and Potter, *Endocrinology*, 2003, 144: 240-246; Silberbach and Roberts, *Cell Signal*, 2001, 13:221-231). The signaling
20 mechanisms underlying ANP's growth regulatory effects are poorly understood, although a number of reports suggest that ANP acts through mitogen-activated protein kinases (Silberbach and Roberts, *Cell Signal*, 2001, 13:221-231).

 Most cells of the mucosal immune system have ANP receptors (NPRs) and there is evidence that natriuretic peptides regulate the immune response and inflammation
25 (Kurihara *et al.*, *Biochem Biophys Res Commun* 1987, 149:1132-1140). ANP stimulates migration of human neutrophils (Izumi *et al.*, *J Clin Invest* 2001, 108:203-213), and inhibit nitric oxide and TNF- α production by murine macrophages (Kierner and Vollmar, *J Biol Chem* 1998, 273:13444-13451; Kierner *et al.*, *J Immunol* 2000, 165:175-81). It has been suggested that the ANP system may be a critical component of the immune response
30 through its actions on both immune and non-immune cells. In patients with lung tumors, the immune response plays a large part in the progression of the disease and, consequently, the NPR system may potentially be involved. The alveolar macrophages in

lung cancer patients secrete more pro-inflammatory cytokines, such as IL-6 and IL-1 β , after LPS stimulation than in persons with non-malignant disease (Matanic *et al.*, *Scand J Immunol* 2003, 57: 173-178). Increased IL-6 in lung cancer patients enhances the acute phase response, and is correlated with poor nutritional status and lowered survival (Martin *et al.*, *Cytokine* 1999, 11; 267-273). The cells of the immune system, such as natural killer (NK) cells, V α 24 NKT, which are necessary for cancer surveillance may also be reduced in lung tumor patients (Motohashi *et al.*, *Int J Cancer* 2002, 102:159-165). The most common clinical paraneoplastic syndrome in patients with small-cell lung cancer (SCLC) is hyponatremia, which is believed to be caused by tumor secretion of vasopressin. Tumor biopsies from patients with SCLC and hyponatremia expressed the gene for ANP (Shimizu *et al.*, *Cancer* 1991, 68: 2284-2288; Bliss *et al.*, *J Natl Can Inst*, 1990, 82: 305-310). Thus, the reduced sodium levels seen in SCLC patients may be attributed to the secretion of ANP (Bliss *et al.*, *J Natl Can Inst*, 1990, 82: 305-310). Human SCLC cell lines express functional ANP receptors (Ohsaki *et al.*, *Cancer Res* 1993, 53: 3165-3171). A majority of SCLC cell lines produce ANP and some produce BNP as well (Ohsaki *et al.*, *Oncology* 1999, 56: 155-159). In contrast, in NSCLC cell lines, which are derived mostly from adenocarcinomas that comprise about two-thirds of all lung cancers, little is known about their growth regulation in response to ANP cascade.

The present inventor has found that the N-terminal natriuretic peptides, such as pNP73-102, are capable of inhibiting NF κ B activation (Mohapatra, international application WO 2004/022003, published March 18, 2004, which is incorporated herein by reference in its entirety), and that the ANP cascade plays a critical role in cell proliferation and inflammation. NF κ B, a transcription factor and a key player in inflammatory processes, has been implicated in the development of cancer in liver and mammary tissues (Greten F.R. *et al. Cell*, 2004, 118: 285-296; Pikarsky E. *et al. Nature*, 2004, 431: 461-466). Activation of the NF- κ B pathway enhances tumor development and may act primarily in the late stages of tumorigenesis. Inhibition of NF- κ B signaling uniformly suppressed tumor development; however, depending upon the model studied, this salutary effect was attributed to an increase in tumor cell apoptosis, reduced expression of tumor cell growth factors supplied by surrounding stromal cells, or abrogation of a tumor cell dedifferentiation program that is critical for tumor invasion/metastasis.

Brief Summary of the Invention

The present invention pertains to methods for reducing natriuretic peptide receptor-A (also known in the art as NPRA, NPR-A, and guanylate cyclase A) activity *in vitro* or *in vivo*. The method of the invention may be used for treating inflammatory and cell proliferation disorders, such as cancer.

In another aspect, the present invention concerns methods for identifying agents useful for treating inflammatory and cell proliferation disorders by determining whether the candidate agent reduces activity of the natriuretic peptide receptor-A (also known in the art as NPRA, NPR-A, and guanylate cyclase A) *in vitro* and/or *in vivo* (also referred to herein as the diagnostic method or assay of the invention).

In another aspect, the method of the present invention may be used for reducing the growth of cancer cells *in vitro* or *in vivo*. In one aspect, the method is useful for treating cancers, such as adenocarcinomas of lung, breast, ovary and melanomas, which may be caused by cell proliferation and inflammation induced by the atrial natriuretic peptide (ANP) cascade.

In one embodiment, the method of the present invention comprises administering a therapeutically effective amount of an agent that reduces NPR-A activity. In another embodiment, the method of the present invention comprises administering a therapeutically effective amount of an N-terminal natriuretic peptide (referred to herein as NP or NP peptide), or a polynucleotide encoding NP and an operably-linked promoter sequence, to a patient in need of such treatment. As used herein, NP refers to peptides derived from atrial natriuretic factor (ANF) hormone, or a biologically active fragment, homolog, or variant thereof. In another embodiment, the method of the present invention comprises administering an effective amount of NP, or a polynucleotide encoding NP and an operably-linked promoter, to one or more cancer cells, wherein the NP is capable of reducing cell proliferation and/or tumor growth. The effect of the NP or a biologically active fragment, homolog, or variant thereof, is capable of reducing cancer cell growth *in vitro* or *in vivo*.

Specifically exemplified NPs comprise an amino acid sequence selected from the group consisting of amino acids 1-30 of ANF (also known as "long acting natriuretic peptide" and referred to herein as NP₁₋₃₀ or SEQ ID NO:1), amino acids 31-67 of ANF (also known as "vessel dilator" and referred to herein as NP₃₁₋₆₇ or SEQ ID NO:2), and

amino acids 79-98 of ANF (also known as "kaliuretic peptide" and referred to herein as NP₇₉₋₉₈ or SEQ ID NO:3), or biologically active fragments or homologs of any of the foregoing. Other exemplified NPs comprise amino acids 73-102 of proANF (referred to herein as NP₇₃₋₁₀₂ or SEQ ID NO:5), or SEQ ID NO:6, or biologically active fragment(s) or homolog(s) of the foregoing. In one embodiment, the NP administered to the patient does not consist of NP₉₉₋₁₂₆ (SEQ ID NO:4).

In another embodiment, the method of the present invention comprises administering an effective amount of at least one nucleic acid molecule encoding an NP to a patient in need of such treatment. The present inventor has determined that introduction of a nucleic acid molecule encoding NP is capable of inhibiting tumor growth and tumor metastasis. The gene delivery method of the present invention permits long-term expression of NP-encoding nucleic acid sequences *in vivo*, thereby conferring anti-cancer effects. In one embodiment, a therapeutically effective amount of at least one nucleic acid molecule encoding a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5 or biologically active fragments or homologs of any of the foregoing, are administered to the patient.

In another aspect, the present invention concerns an isolated peptide comprising the amino acid sequence NP₇₃₋₁₀₂ (SEQ ID NO:5) or SEQ ID NO:6, or a biologically active fragment or homolog of the foregoing. In another aspect, the present invention concerns an isolated nucleic acid molecule encoding the amino acid sequence of NP₇₃₋₁₀₂ (SEQ ID NO:5) or encoding the amino acid sequence of SEQ ID NO:6, or a biologically active fragment or homolog thereof.

In another aspect, the present invention concerns an expression vector comprising a nucleic acid sequence encoding an NP, and a promoter sequence that is operably linked to the NP-encoding nucleic acid sequence. In one embodiment, the expression vector is a DNA plasmid or virus. In another aspect, the present invention concerns a pharmaceutical composition comprising a nucleic acid sequence encoding an NP, and a pharmaceutically acceptable carrier.

Brief Description of the Drawings

For a fuller understanding of the nature and objects of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

5 **Figure 1** shows pNP 73-102 inhibits NPRA expression. Pregnant (12 days) mice were injected i.p. with pVAX (vector), or pNP73-102. After 1 day, mice were sacrificed, thymi removed from the embryo, and homogenized. Cells were centrifuged and erythrocytes were lysed and incubated with anti-NPR-Ab or anti-NPR-C for 1 hour, washed, and incubated with PE-conjugated secondary antibodies. Levels of NPRA and
10 NPRC were determined by flow cytometry.

Figures 2A-2D show NPRA deficiency decreases pulmonary inflammation. Groups (n=3) of wild type DBA/2 (wt) (Figure 2A) and NPR-C deficient (NPRC^{-/-}) (Figure 2B) mice and wild type C57/BL6 (wt) (Figure 2C) and NPR-A (NPRA^{-/-}) (Figure 2D) were sensitized with OVA (20mg/mouse) and after 2 weeks challenged i.n. with
15 OVA (20mg/mouse). One day later mice were sacrificed and lung sections were stained with H & E to examine inflammation.

Figures 3A-3D demonstrate that A549 cells transfected with pNP₇₃₋₁₀₂ show a significantly higher level of apoptosis compared to control and pANP or pVAX (Figures 3A-3C). Cells were transfected with pNP73-102, pANP and pVAX (as control) and cells
20 were stained with PI and annexin and quantified by flow cytometry (Figure 3D). The proteins were isolated and an equal amount of the cell lysates were western-blotted using an antibody to poly-ADP ribose polymerase (PARP). The results demonstrate that pNP73-102 shows a higher accumulation of apoptotic cells compared to cells transfected with pANP and pVAX controls.

25 **Figure 4** shows that pNP73-102 decreases tumorigenesis in a colony formation assay by A549. Six centimeter tissue culture plates were covered with 4 ml of 0.5% soft agar. A549 cells were transfected with pANP, pNP₇₃₋₁₀₂ and pVAX plasmid DNA (V) or nothing (C). After 40 h of transfection, cells were suspended in 2 ml of 0.3% soft agar and added to each plate. Cells were plated in duplicate at a density of 2×10^4 cells /dish
30 and incubated for two weeks. Plates were photographed under a microscope. Cell colonies were counted and plotted. The results of one representative experiment of two is shown.

Figures 5A-5E show expression of NP₇₃₋₁₀₂-FLAG in the BAL cells after i.n. administration of chitosan encapsulated plasmid pNP₇₃₋₁₀₂-FLAG construct. BAL was performed in mice (n=3) after 24 hours and BAL cells were stained with either the second antibody control or anti-FLAG antibody (SIGMA) and then with DAPI. A representative staining is shown (Figures 5A-5C). Figure 5D shows lungs removed from mice treated with chitosan nanoparticles carrying pNP₇₃₋₁₀₂ (CPNP73-102) (Rx) or empty plasmid pVAX (control). The lungs of control mice showed several lung nodules in contrast to mice treated with CPNP73-102, which showed very few tumors. Intranasal CPNP73-102 administration abrogated tumor formation in A549 injected nude mice. Nude mice were given 5×10^6 cells intravenously (tail vein) and weekly injections of nanoparticle carrying either empty plasmid (control) or pNP73-102 (Rx). Three weeks later, mice were sacrificed and lung sections were stained with H & E to examine the lung nodules (Figure 5D). Control shows nodules and tumor cell mass, whereas the treated group had no tumors. Sections were also stained with antibodies to cyclinB and to phospho-Bad (Figure 5E). The results show that mice treated with CPNP73-102 had no tumors in the lung and did not show any staining for pro-mitotic Cyclin-B and anti-apoptotic marker phospho-Bad.

Figures 6A-6D demonstrate that treatment with chitosan nanoparticles carrying pNP₇₃₋₁₀₂ (CPNP73-102) decreases the tumor burden in a spontaneous tumorigenesis model of immunocompetent BALB/c mice. Two groups of mice (n=4) were administered with the Line-1 tumor cells (100,000 cells/mouse) at the flanks. One group was administered with CPNP73-102 the same day, whereas another group was administered with vehicle alone (nanoparticle carrying a plasmid without NP73-102) and the third group was given the saline. Treatment was continued with CPNP73-102 or control at weekly intervals for 5 weeks. The tumors were dissected out from the mice of each group (Figures 6A-6C) and the tumor burden was calculated by weighing them on a balance and expressed as tumor mass per g lung weight. Results are shown in Figure 6D.

Figure 7 shows that CPNP73-102 induces apoptosis in chemoresistant ovarian cancer cells. C-13 and OV2008 ovarian cancer cells were transfected with pNP73-102. Forty-eight hours later, cells were processed for TUNEL assay to examine apoptosis. The results of one of two representative experiments are shown.

Figure 8 shows breast cancer MCF-7 cell counts. The cells were transfected with pVAX, pANP, and pANP₇₃₋₁₀₂ and counted at 24 and 48 hours after transfection. 30 ml

of Trypan Blue was mixed with 30 ml for measuring the cell viability. The results of one of two representative experiments are shown.

Brief Description of the Sequences

- 5 SEQ ID NO:1 is the amino acid sequence of human "long acting natriuretic peptide" or NP₁₋₃₀: ¹NPMYN AVSNADLMDF KNLLDHLEEK MPLED³⁰ (SEQ ID NO:1).

SEQ ID NO:2 is the amino acid sequence of human "vessel dilator" or NP₃₁₋₆₇:
³¹EVVPP QVLSEPNEEA GAALSPLPEV PPWTGEVSPA QR⁶⁷ (SEQ ID NO:2).

10

SEQ ID NO:3 is the amino acid sequence of human "kaliuretic peptide" or NP₇₉₋₉₈:
⁷⁹SSDRSAL LKSKLRALLT APR⁹⁸ (SEQ ID NO:3).

- 15 SEQ ID NO:4 is the amino acid sequence of human "atrial natriuretic peptide" (ANP) or NP₉₉₋₁₂₆: ⁹⁹SLRRSSC FGGRMDRIGA QSGLCNSFR Y¹²⁶ (SEQ ID NO:4).

SEQ ID NO:5 is the amino acid sequence of cloned mouse pNP₇₃₋₁₀₂:
⁷³GSPWDPSDRS ALLKSKLRAL LAGPRSLRR¹⁰² (SEQ ID NO:5).

- 20 SEQ ID NO:6 is the amino acid sequence of cloned mouse NP fragment:
 VSNTDLMDFK NLLDHLEEKM PVEDEVMPQ ALSEQTE (SEQ ID NO:6).

- 25 SEQ ID NO:7 is the amino acid sequence for the human preproANP (NCBI ACCESSION # NM_006172) wherein the underlined amino acids represent the signal sequence which is cleaved off to form the mature peptide:
¹MSSFSTTTVS FLLLLAFOLL GOTRANNPMYN AVSNADLMDF KNLLDHLEEK
 MPLEDEVVPP QVLSEPNEEA GAALSPLPEV PPWTGEVSPA QRDGGALGRG
 PWDSSDRSAL LKSKLRALLT APRSLRRSSC FGGRMDRIGA QSGLCNSFR Y¹⁵¹
 (SEQ ID NO:7).

30

SEQ ID NO:8 is a forward primer for the cDNA sequence encoding mouse prepro ANF protein:

5'- gac ggc aag ctt act atg ggc agc ccc tgg gac cc-3' (SEQ ID NO:8).

SEQ ID NO:9 is a reverse primer for the cDNA sequence encoding mouse pre-proANF protein:

5'- acc ccc ctc gag tta tta tct tcg tag gct ccg-3' (SEQ ID NO:9).

5

SEQ ID NO:10 is a forward primer for the cDNA sequence encoding mouse NP fragment:

5'-aat cct aag ctt agt atg gtg tcc aac aca gat-3' (SEQ ID NO:10).

10 SEQ ID NO:11 is a reverse primer for the cDNA sequence encoding mouse NP fragment:

5'- tgc gaa ctc gag tta ctc agt ctg ctc act cag ggc ctg cg-3' (SEQ ID NO:11).

SEQ ID NO:12 is the nucleotide sequence encoding cloned mouse pNP₇₃₋₁₀₂:

atg ggc agc ccc tgg gac ccc tcc gat aga tct gcc ctc ttg aaa agc aaa ctg agg gct ctg ctc gct

15 ggc cct cgg agc cta cga aga taa (SEQ ID NO:12).

SEQ ID NO:13 is the nucleotide sequence encoding cloned mouse pNP fragment:

atg gtg tcc aac aca gat ctg atg gat ttc aag aac ctg cta gac cac ctg gag gag aag atg ccg gta

gaa gat gag gtc atg ccc ccg cag gcc ctg agt gag cag act gag taa (SEQ ID NO:13).

20

SEQ ID NO:14 is the mRNA nucleotide sequence encoding human ANP (NCBI Accession # NM_006172:

1 tggcgaggga cagacgtagg ccaagagagg ggaaccagag aggaaccaga ggggagagac

61 agagcagcaa gcagtggatt gctccttgac gacgccagca tgagctcctt ctccaccacc

25 121 accgtgagct tcctcctttt actggcattc cagctcctag gtcagaccag agctaattcc

181 atgtacaatg ccgtgtccaa cgcagacctg atggatttca agaatttgct ggaccatttg

241 gaagaaaaga tgcctttaga agatgaggtc gtgccccac aagtgtcag tgagccgaat

301 gaagaagcgg gggctgctct cagccccctc cctgaggtgc ctccctggac cggggaagtc

361 agcccagccc agagagatgg aggtgccctc gggcgggg cc cctgggactc ctctgatcga

30 421 tctgccctcc taaaaagcaa gctgagggcg ctgctcactg ccctcggag cctgcggaga

481 tccagctgct tcgggggcag gatggacagg attggagccc agagcggact gggctgtaac

541 agcttccggt actgaagata acagccaggg aggacaagca gggctggg cc tagggacaga

601 ctgcaagagg ctctgtccc ctggggtctc tgctgcattt gtgtcatctt gttgcatgg

661 agttgtgac atcccatcta agctgcagct tctgtcaac acttctcaca tcttatgcta
 721 actgtagata aagtggtttg atggtgactt cctcgcctct cccaccccat gcattaaatt
 781 ttaaggtaga acctcacctg ttactgaaag tggtttgaag gtgaataaac ttcagcacca
 841 tggac (SEQ ID NO:14).

5

SEQ ID NO:15 is the human gene for atrial natriuretic factor propeptide (coding sequence includes - join (570...692, 815...1141, 2235...2240); sig. peptide = 570...644; mat. peptide = join (645...692, 815...1141, 2235...2237), (NCBI ACCESSION NO: X01471; Greenberg, B.D. *et al.*, *Nature*, 1984, 312(5995):656-658):

10 1 ggatccattt gtctcgggct gctggctgcc tgccattcc tcctctccac ccttatttgg
 61 aggccctgac agctgagcca caaacaacc aggggagctg ggcaccagca agcgtcaccc
 121 tctgtttccc cgcacgttac cagcgtcgag gagaagaat cctgaggcac ggcggtgaga
 181 taaccaagga ctcttttta ctctctcac accttgaag tgggagcctc ttgagtcaaa
 241 tcagtaagaa tgcggctctt gcagctgagg gtctgggggg ctgttggggc tgcccaaggc
 15 301 agagaggggc tgtgacaagc cctgcggatg ataacttaa aaggcatct cctgctggct
 361 tctcatttgg cagctttatc actgcaagt acagaatggg gagggttctg tctctcctgc
 421 gtgcttggag agctgggggg ctataaaaag aggcggcact gggcagctgg gagacaggga
 481 cagacgtagg ccaagagagg ggaaccagag aggaaccaga ggggagagac agagcagcaa
 541 gcagtggatt gtccttgac gacgccagca tgagctcctt ctccaccacc accgtgagct
 20 601 tcctccttt actggcattc cagctcctag gtcagaccag agctaattcc atgtacaatg
 661 ccgtgtccaa cgcagacctg atggatttca aggtagggcc aggaaagcgg gtgcagtctg
 721 gggccagggg gctttctgat gctgtgctca ctctcttga ttctctcaa gtcaagtagg
 781 ttatccctt tcctgtatt ttcttttct aaagaatttg ctggaccatt tggaagaaaa
 841 gatgccttta gaagatgagg tcgtccccc acaagtgtc agtgagccga atgaagaagc
 25 901 gggggctgct ctacgcccc tcctgaggt gcctccctgg accggggaag tcagcccagc
 961 ccagagagat ggaggtgccc tcgggcgggg ccctgggac tcctctgac gatctgcct
 1021 ctaaaaaagc aagctgaggg cgctgtcac tgcccctcgg agcctgcgga gatccagctg
 1081 ctccgggggc aggatggaca ggattggagc ccagagcggga ctgggctgta acagcttccg
 1141 ggtaagagga actggggatg gaaatgggat gggatggaca ctactgggag acaccttcag
 30 1201 caggaaagg accaatgcag aagctcatt cctctcaagt ttctgcccc acaccagag
 1261 tgcccatggt gtgtcaggac atgcatcta ttgtcttag ctagtctgt gagaaatgc
 1321 ttaaaaaaaa aagggggggg gctgggcacg gtcgtcacgc ctgtaatccc agcactttgg
 1381 gaggccaggc agcggatcat gaggtcaaga gatcaagact atcctggcca acatggtgaa

1441 accccagctc tactaaaaat acaaaaatta gctgggtgtg tggcgggcac ctgtactctc
 1501 agctacttgg gaggtgagg caggagaatc actgaacc aggaggcaga ggttgagtg
 1561 agcagagatc acgccactgc agtcagcct aggtgataga gcgagactgt ctcaaaaaaa
 1621 aaaaaaaaag gccaggcgcg gtggctcag cctgtaatcc cagcgcttg ggaggccaag
 5 1681 gcgggtggat cacgaggtca ggagatggag accatcctgg ctaacacggt gaaaccccgt
 1741 ctctactaaa aatacaaaaa attagccagg cgtgggtggca ggcgctgta agtcctagct
 1801 actccggagg ctgaggcagg agaattggcgt gaaccggga ggcggagctt gcagttagca
 1861 gagatggcac cactgcactc cagcctgggc gacagagcaa gactccgtct caaaaaaaaa
 1921 aaaaaaaaaa gcaactgcc atagcactgg gaaataaaa tattcataga gccaaagtat
 10 1981 ctttgcattg ctgattagca gtcatattc ctcccagaa ttgcaagatc ctgaagggtc
 2041 taagtgaat ttactctgat gagtaacttg cttatcaatt catgaagctc agagggtcat
 2101 caggctgggg tgggggccgg tgggaagcag gtggtcagta atcaagtca gaggatgggc
 2161 aactcatac atgaagtga ctttccagg acagccaggt caccaagcca gatatgtctg
 2221 tgttctctt gcagtactga agataacagc caggaggac aagcagggtt gggcctaggg
 15 2281 acagactgca agaggctcct gtcccctggg gtctctgctg catttgtgc atcttgtgc
 2341 catggagttg tgatcatccc atctaagctg cagcttctg tcaacacttc tcaatctta
 2401 tgctaactgt agataaagt gttgatggg gacttctcg cctctccac cccatgcatt
 2461 aaattttaag gtagaacctc acctgttact gaaagtgggt tgaaagtga taaacttcag
 2521 caccatggac agaagacaaa tgctgcgtt ggtgtgctt cttctctt gggaagagaa
 20 2581 ttc (SEQ ID NO:15).

SEQ ID NO:16 is the amino acid sequence for the mouse preproANP peptide:

MGSFSITLGF FLVLAFWLPG HIGANPVYSA VSNTDLMDFK NLLDHLEEKM
 PVEDEVMP PQ ALSEQTEEAG AALSSLPEVP PWTGEVNPL RDGSALGRSP
 25 WPSDRSALL KSKLRALLAG PRSLRRSSCF GGRIDRIGAQ SGLGCNSFRY RR
 (SEQ ID NO:16).

SEQ ID NO: 17 is the genetic sequence for the mouse preproANP peptide wherein the
 coding sequence starts at nucleic acid molecule position 81 and ends at nucleic acid
 30 molecule position 539:

1 caaaagctga gagagagaga gaaagaaacc agagtgggca gagacagaa acatcagatc
 61 gtgccccgac ccacgccagc atgggtcctt tctccatcac cctgggcttc ttcctcgtct
 121 tggccttttg gcttcaggc catattggag caaatcctgt gtacagtgcg gtgtccaaca

181 cagatctgat ggatttcaag aacctgctag accacctgga ggagaagatg ccggtagaag
241 atgaggtcat gccccgcag gccctgagtg agcagactga ggaagcaggg gccgcactta
301 gctccctccc cgaggtgcct ccctggactg gggaggtcaa cccacctctg agagacggca
361 gtgctctagg gcgcagcccc tgggaccct cccatagatc tgcctcttg aaaagcaaac
5 421 tgagggtctt gctcgtggc cctcggagcc tacgaagatc cagctgctt ggggtagga
481 ttgacaggat tggagcccag agtggactag gctgaacag ctccggtac cgaagataac
541 agccaaggag gaaaaggcag tcgattctgc ttgagcagat cgcaaaagat cctaagccct
601 tgtggtgtgt caccgagctt ggacacattg ccactgtggc gtggtgaaca cctcctgga
661 gctgcggctt cctgccttca tctatcacga tcgatgtaa atgtagatga gtggtctagt
10 721 ggggtcttgc ctctccact ctgcatatta agtagatcc tcacccttt cagaaagcag
781 ttggaaaaaa aaaaaagaa taaacttcag caccaaggac agacgccgag gccctgatgt
841 gcttcttgg cttctgcct cagtcttgg ctctccc (SEQ ID NO:17).

SEQ ID NO:18 is amino acid sequence of human natriuretic peptide receptor-A (NPR-A):

15 MPGPRRPAGSRLRLLLLLLLPLLLLLRGSHAGNLTAVVLPLANTSYPWSWAR
VGPAVELALAQVKARPDLLPGWTVRTVLGSSSENALGVCSDTAAPLAAVDLKWE
HNPAVFLGPGCVYAAPVGRFTAHWRVPLLTAGAPALGFGVKDEYALTTRAGP
SYAKLGDFVAALHRRLGWERQALMLYAYRPGDEEHCFLLVEGLFMRVRDRLNI
TVDHLEFAEDDLSHYTRLLRTMPRKGRVIYICSSPDAFRTLMLLALEAGLCGEDY
20 VFFHLDIFGQSLQGGQGPAPRRPWERGQDV SARQAFQAAKIITYKDPDNPEYL
EFLKQLKHLAYEQFNFTMEDVLVNTIPASFHDGLLLYIQAVTETLAHGGTVTDGE
NITQRMWNRSFQGV TGYLKIDSSGDRETDFSLWDMDPENGAFRVVLNYNGTSQ
ELVAVSGRKLNWPLGYPPPDIPKCGFDNEDPACNQDHLSTLEVLALVGSLSLLGI
LIVSFFIYRKMQLEKELASELWRVRWEDVEPSSLERHLRSAGSRLTLSGRGSNYG
25 SLLTTEGQFQVFAKTAYYKGNLVAVKRVNRKRIELTRKVL FELKHM RDVQNEH
LTRFVGACTDPPNICILTEYCPRGSLQDILENESITLDWMFRYSLTNDIVKGMLFL
HNGAICSHGNLKSSNCVVDGRFVLKITDYGLESRDLDP EQGHTVYAKKLWTAP
ELLRMASPPVRGSQAGDVYSFGILQEIALRSGVFHVEGLDLSPKEI IERVTRGEQP
PFRPSLALQSHLEELGLLMQRCWAEDPQERPPFQQIRLTLRKFNRENS SNILDNLL
30 SRMEQYANNLEELVEERTQAYLEEKRKAEALLYQILPHSVAEQLKRGETVQAEA
FDSVTIYFSDIVGFTALSAESTPMQVVTLLNDLYTCFDAVIDNFDVYKVETIGDAY
MVVSGLPVRNGRLHACEVARMALALLDAVRSFRIRHRPQEQLRLRIGIHTGPVC

AGVVGLKMPRYCLFGDTVNTASRMESNGEALKIHLSSSETKAVLEEFGGFELELR
GDVEMKGKGKVRTYWLLGERGSSTRG (SEQ ID NO:18).

(NCBI ACCESSION NO. NM_000906; Airhart N. *et al.*, *J. Biol. Chem.*, 2003, 278(40):38693-38698; Pitzalis M.V. *et al.*, *J. Hypertens.*, 2003, 21(8):1491-1496; Mokentin J.D. *J. Clin. Invest.*, 2003, 111(9):1275-1277; De L. *et al.*, *J. Biol. Chem.*, 2003, 278(13):11159-11166; Knowles J.W. *et al.*, *Hum. Genet.*, 2003, 12(1):62-70; Pandey K.N. *et al.*, *J. Biol. Chem.*, 2002, 277(7):4618-4627).

10 SEQ ID NO:19 is the nucleotide coding sequence for human natriuretic peptide receptor-A (NPR-A):

ggttccctcc ggatagccgg agacttgggc cggccggacg ccccttctgg cacactccct
61 ggggcaggcg ctacgcacg ctacaaacac aactctctct ttctccctc gcgcgcctc
121 tctcactctt cttcacgaag cgctcactcg caccctttct ctctctctct ctctctctaa
15 181 cagcgcacga cactccaggt tgttcacact cgggtcctct ccagcccgac gttctcctgg
241 caccacactg ctccgcgggc cctgcgcgc cccctcgggt cgcgcctctt gcgctctcgg
301 cccagaccgt cgcagctaca gggggcctcg agccccgggg tgagcgtccc cgtcccgtc
361 ctgctccttc ccatagggac gcgcctgatg cctgggaccg gccgctgagc ccaaggggac
421 cgaggaggcc atggtaggag cgctcgctg ctgcggtgcc cgctgaggcc atgccggggc
20 481 cccggcgccc cgttggtctc cgctgcgc tctctctgct cctgtgctg ccgcccgtgc
541 tgtgtctgct ccggggcagc cagcgggca acctgacggt agcgtggta ctgccgtgg
601 ccaatactc gtacccctgg tctggggcgc gcgtgggacc cgcgtggag ctggccctgg
661 cccaggtgaa ggcgcgcccc gacttctgc cgggtggac ggtccgcac gtgtgggca
721 gcagcgaaaa cgcgtgggc gtctgtccg acaccgcagc gccctggcc gcggtggacc
25 781 tcaagtggga gcacaaccc gctgtgttc tgggccccgg ctgcgtgtac gccgccgccc
841 cagtggggcg cttaccgcg cactggcggg tcccgtgct gaccgccgc gccccggcgc
901 tgggcttcgg tgtcaaggac gagtatgcgc tgaccaccg cgcggggccc agctacgca
961 agctggggga cttcgtggcg gcgtgcacc gacggctggg ctgggagcgc caagcgtca
1021 tgtctacgc ctaccggccg ggtgacgaag agcactgctt cttctcgtg gaggggctgt
30 1081 tcattcggtt ccgcgaccgc ctcaatatta cgggtggacca cctggagttc gccgaggacg
1141 acctcagcca ctacaccagg ctgtgcgga ccatgccgcg caaaggccga gttatctaca
1201 tctgcagctc ccctgatgcc ttcagaacct tcattgctct ggccctggaa gctggctgt
1261 gtggggagga ctacgtttc ttccactgg atatcttgg gcaaagcctg caaggtggac
1321 agggccctgc tccccgcagg ccttgggaga gaggggatgg gcaggatgtc agtccccgcc
35 1381 aggccttca ggctgcaaaa atcattacat ataaagacc agataatccc gactactgg
1441 aattcctgaa gcagttaaaa cactggcct atgagcagtt caattcacc atggaggatg
1501 tcttggtgaa caccatccca gcatccttc acgacgggt cctgtctat atccaggacg
1561 tgacggagac tctggcacat gggggaactg ttactgatgg ggagaacatc actcagcgga
1621 tgtggaaccg aagcttcaa ggtgtgacag gatactgaa aattgatagc agtggcgatc
40 1681 gggaacaga cttctccctc tgggatatgg atcccagaa tggcgcttc aggggtgtac
1741 tgaactaaa tgggacttcc caagagctgg tggctgtgc ggggcgcaaa ctgaactggc
1801 ccctggggta cctcctcct gacatccca aatgtggctt tgacaacga gaccagcat
1861 gcaaccaaga tcactttcc accctggagg tctggcttt ggtgggcagc ctctcctgc
1921 tcggcattct gattgtctc ttctcatat acaggaagat gcagctggag aaggaactgg

1981 cctcggagct gtggcgggtg cgctgggagg acgttgagcc cagtagcctt gagaggcacc
 2041 tgcggagtgc aggagccgg ctgaccctga gcgggagagg ctccaattac ggctccctgc
 2101 taaccacaga gggccagttc caagtcttg ccaagacagc atattataag ggcaacctcg
 2161 tggctgtgaa acgtgtgaac cgtaaacgca ttgagctgac acgaaaagtc ctgtttgaac
 5 2221 tgaagcatat gcgggatgtg cagaatgaac acctgaccag gtttgtggga gcctgcaccg
 2281 acccccccaa tatctgcatc ctacagagt actgtccccg tgggagcctg caggacattc
 2341 tggagaatga gagcatcacc ctggactgga tgtccggtta ctactcacc aatgacatcg
 2401 tcaaggggcat gctgtttcta cacaatgggg ctatctgttc ccatgggaac ctcaagtc
 2461 ccaactgcgt ggtagatggg cgctttgtgc tcaagatcac cgactatggg ctggagagct
 10 2521 tcaggggacct ggaccagag caaggacaca ccgtttatgc caaaaagctg tggacggccc
 2581 ctgagctcct gcgaatggct tcaacccctg tgcggggctc ccaggctggt gacgtataca
 2641 gctttgggat catcctcag gagattgccc tgaggagtgg ggtcttcac gtggaaggtt
 2701 tggacctgag ccccaaagag atcatcgagc gggtgactcg gggtgagcag ccccccttc
 2761 ggccctccct ggccctgcag agtcacctgg aggagtggg gctgctcatg cagcgggtct
 15 2821 gggctgagga cccacaggag agggccaccat tccagcagat ccgcctgacg ttgcgcaat
 2881 ttaacaggga gaacagcagc aacatcctgg acaacctgct gtcccgcag gagcagtacg
 2941 cgaacaatct ggaggaaactg gtggaggagc ggaccagggc atacctggag gagaagcgca
 3001 aggtgagggc cctgctctac cagatcctgc ctactcagt ggctgagcag ctgaagcgtg
 3061 gggagacggt gcagggcgaa gcctttgaca gtgttaccat ctacttcagt gacattgtgg
 20 3121 gtttcacagc gctgtcggcg gagagcagc ccatgcaggt ggtgaccctg ctcaatgacc
 3181 tgtacacttg cttgatgt gtcatagaca actttgatgt gtacaagggt gagacaattg
 3241 gcgatgcta catggtggtg tcaagggtcc ctgtgcggaa cgggcgggta cacgcctgcg
 3301 aggtagcccg catggccctg gcaactgctg atgtgtgcg ctcttccga atccgccacc
 3361 ggccccagga gcagctgcgc ttgcgcattg gcatccacac aggacctgtg tgtctggag
 25 3421 tgggtgggact gaagatgccc cgcttactgt tctttgggga tacagtcaac acagcctcaa
 3481 gaatggagtc taatggggaa gcctgaaga tccactgtc ttctgagacc aaggctgtcc
 3541 tggaggagtt tgggtgttc gagctggagc ttcgagggga ttagaaaatg aagggcaaag
 3601 gcaagggtcg gacctactgg ctcttgggg agagggggag tagcaccgga ggctgacctg
 3661 cctcctcc tatectcca cactccct accctgtgcc agaagcaaca gaggtgccag
 30 3721 gcctcagcct caccacagc agccccatcg ccaaaggatg gaagtaattt gaatagctca
 3781 ggtgtgctta cccagtgaa gacaccagat aggacctctg agaggggact ggcatggggg
 3841 gatctcagag cttacaggct gagccaagcc cacggccatg cacagggaca ctacacagg
 3901 cacacgcacc tctctccac ctggactcag gccgggctgg gctgtggatt cctgatcccc
 3961 tcccccccc atgtctct cctcagcct tctaccctg tgacttactg ggaggagaaa
 35 4021 gagtacactg aagggggaaca tgaagagaga ctaggtgaag agagggcagg ggagcccaca
 4081 tctggggctg gccacaata cctgtcccc cgacccctc caccagcag tagacacagt
 4141 gcacagggga gaagaggggt ggcgcagaag ggttgggggc ctgtatgcct tgctctacc
 4201 atgagcagag acaattaaaa tctttattcc aaaaaaaaaa aaaaaa (SEQ ID NO:19)

40 (NCBI ACCESSION NO. NM_000906; Airhart N. *et al.*, *J. Biol. Chem.*, 2003,
 278(40):38693-38698; Pitzalis M.V. *et al.*, *J. Hypertens.*, 2003, 21(8):1491-1496;
 Mokentin J.D. *J. Clin. Invest.*, 2003, 111(9):1275-1277; De L. *et al.*, *J. Biol. Chem.*, 2003,
 278(13):11159-11166; Knowles J.W. *et al.*, *Hum. Genet.*, 2003, 12(1):62-70; Pandey K.N.
et al., *J. Biol. Chem.*, 2002, 277(7):4618-4627).

SEQ ID NO:20 is amino acid sequence of the human atrial natriuretic peptide clearance receptor precursor (ANP-C; also referred to as NPR-C, NPRC, and atrial natriuretic peptide C-type receptor):

MPSLLVLTFS PCVLLGWALL AGGTGGGGVG GGGGGAGIGG GRQEREALPP
 5 QKIEVLVLLP QDDSYLFSLT RVRPAIEYAL RSVEGNGTGR RLLPPGTRFQ
 VAYEDSDCGN RALFSLVDRV AAARGAKPDL ILGPVCEYAA APVARLASHW
 DLPMLSA GAL AAGFQHKDSE YSHLTRVAPA YAKMGEMMLA LFRHHHWSRA
 ALVYSDDKLE RNCYFTLEGV HEVFQEEGLH TSIYSFDETK DLDLEDIVRN
 IQASERVVIM CASSDTIRSI MLVAHRHGMT SGDYAFFNIE LFNSSSYGDG
 10 SWKRGDKHDF EAKQAYSSLQ TVTLLRTVKP EFEKFSMEVK SSVEKQGLNM
 EDYVNMFVEG FHDAILLYVL ALHEVLRAGY SKKDGGKIIQ QTNWRTFEGI
 AGQVSIDANG DRYGDFS VIA MTDVEAGTQE VIGDYFGKEG RFEMRPNVKY
 PWGPLKLRIE ENRIVEHTNS SPCKSSGGLE ESAVTGIVVG ALLGAGLLMA
 FYFFRKKYRI TIERRTQQEE SNLGKHXREL EDSIRSHFSV A (SEQ ID NO:20)
 15 (NCBI ACCESSION NO. P17342; Lowe D.G. *et al.*, *Nucleic Acids Res.*, 1990,
 18(11):3412; Porter J.G. *et al.*, *Biochem. Biophys. Res. Commun.*, 1990, 171(2):796-803;
 Stults J.T. *et al.*, *Biochemistry*, 1994, 33(37):11372-11381).

SEQ ID NO:21 is an siRNA specific for NPR-A (human).

20 tat tac ggt gga cca cct gtt caa gag aca ggt ggt cca ccg taa tat tttt

SEQ ID NO:22 is an siRNA specific for NPR-A (human).

aga att cca gaa acg cag ctt caa gag agc tgc gtt tct gga att ctt tttt

25 Detailed Disclosure

The present invention pertains to methods for reducing natriuretic peptide
 receptor-A (also known in the art as NPRA, NPR-A, and guanylate cyclase A) activity *in*
vitro or *in vivo*. In one aspect, the method of the invention may be used for treating
 inflammatory and cell proliferation disorders, such as cancer. In another aspect, the
 30 present invention concerns methods for identifying agents useful for treating
 inflammatory and cell proliferation disorders by determining whether the candidate agent
 reduces activity of the natriuretic peptide receptor-A (also known in the art as NPRA,

NPR-A, and guanylate cyclase A) *in vitro* and/or *in vivo* (also referred to herein as the diagnostic method or assay of the invention).

As used herein, an "inflammatory disorder" includes those conditions characterized by an aberrant increase in one or more of the following: IL-6, IL-1 beta, TNF-alpha, IL-8, eosinophil production, neutrophil production, release of histamines, proliferants, hyperplasia, and cell adhesion molecule expression. As used herein, a "cell proliferation disorder" is characterized by one or more of the following: uncontrolled proliferation, a high mitogenic index, over-expression of cyclin D1, cyclin B1, expression of an oncogene such as c-jun and/or c-fos, aberrant activation of NFkB and/or ERK (extracellular receptor kinase), and matrix metalloproteinase expression (such as MMP-2 and/or MMP-9).

In one embodiment, the inflammatory disorder and cell proliferation disorder is not one that is amenable to effective treatment by administration of a vasodilator. In one embodiment, the inflammatory disorder and cell proliferation disorder is not a cardiovascular disorder (such as hypertension or stroke). In another embodiment, the inflammatory disorder and cell proliferation disorder is not a disorder of the central nervous system (such as Alzheimer's disease or other dementia). In another embodiment, the inflammatory disorder and cell proliferation disorder is not kidney failure or other kidney disorder.

The agent used to reduce NPR-A activity *in vitro* or *in vivo* can be virtually any substance and can encompass numerous chemical classes, including organic compounds or inorganic compounds. Preferably, an effective amount of the agent is administered to the cells with a pharmaceutically acceptable carrier. The agent may be a substance such as genetic material, protein, lipid, carbohydrate, small molecules, a combination of any of two or more of foregoing, or other compositions. The agent may be naturally occurring or synthetic, and may be a single substance or a mixture. The agent can be obtained from a wide variety of sources including libraries of compounds. The agent can be or include, for example, a polypeptide, peptidomimetic, amino acid(s), amino acid analog(s), function-blocking antibody, polynucleotide(s), polynucleotide analog(s), nucleotide(s), nucleotide analog(s), or other small molecule(s). A polynucleotide may encode a polypeptide that potentially reduces NPR-A activity within the cell, or the polynucleotide may be a short interfering RNA (siRNA), a hairpin RNA (shRNA), antisense oligonucleotide, ribozyme, or other polynucleotide that targets an endogenous or

exogenous gene for silencing of gene expression and potentially NPR-A activity within the cell.

In one embodiment, the agent used to reduce NPR-A activity is an interfering RNA specific for NPR-A mRNA, preferably human NPR-A mRNA. Interfering RNA is capable of hybridizing with the mRNA of a target gene and reduce and/or eliminate translation through the mechanism of RNA interference. Examples of such interfering RNA include SEQ ID NO:21 and SEQ ID NO:22, which were determined to have a relatively high probability of reducing NPR-A activity using an siRNA Target Finder program (AMBION) and in accordance with published guidelines (Tuschl T., *Nature Biotechnol.*, 2002, 20:446-448). As used herein, the term "RNA interference" ("RNAi") refers to a selective intracellular degradation of RNA. RNAi occurs in cells naturally to remove foreign RNAs (e.g., viral RNAs). Natural RNAi proceeds via fragments cleaved from free dsRNA which direct the degradative mechanism to other similar RNA sequences. Alternatively, RNAi can be initiated by the hand of man, for example, to silence the expression of target genes.

As used herein, the term "small interfering RNA" ("siRNA") (also referred to in the art as "short interfering RNAs") refers to an RNA (or RNA analog) that is capable of directing or mediating RNA interference. In one embodiment, the siRNA is between about 10-50 nucleotides (or nucleotide analogs). Optionally, a polynucleotide (e.g., DNA) encoding the siRNA may be administered to cells *in vitro* or *in vivo*, such as in a vector, wherein the DNA is transcribed.

As used herein, a siRNA having a "sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi)" means that the siRNA has a sequence sufficient to trigger the destruction of the target mRNA by the RNAi machinery or process. "mRNA" or "messenger RNA" or "transcript" is single-stranded RNA that specifies the amino acid sequence of one or more polypeptides. This information is translated during protein synthesis when ribosomes bind to the mRNA.

The scientific literature is replete with reports of endogenous and exogenous gene expression silencing using siRNA, highlighting their therapeutic potential (Gupta, S. *et al. PNAS*, 2004, 101:1927-1932; Takaku, H. *Antivir Chem. Chemother*, 2004, 15:57-65; Pardridge, W.M. *Expert Opin. Biol. Ther.*, 2004, 4:1103-1113; Zheng, B.J. *Antivir. Ther.*, 2004, 9:365-374; Shen, W.G. *Chin. Med. J. (Engl)*, 2004, 117:1084-1091; Fuchs, U. *et al. Curr. Mol. Med.*, 2004, 4:507-517; Wadhwa, R. *et al. Mutat. Res.*, 2004, 567:71-84;

Ichim, T.E. *et al.* *Am. J. Transplant*, 2004, 4:1227-1236; Jana, S. *et al.* *Appl. Microbiol. Biotechnol.*, 2004, 65:649-657; Ryther, R.C. *et al.* *Gene Ther.*, 2005, 12:5-11; Chae, S-S. *et al.*, *J. Clin. Invest.*, 2004, 114:1082-1089; Fougerolles, A. *et al.*, *Methods Enzymol.*, 2005, 392:278-296), each of which is incorporated herein by reference in its entirety.

5 Therapeutic silencing of endogenous genes by systemic administration of siRNAs has been described in the literature (Kim B. *et al.*, *American Journal of Pathology*, 2004, 165:2177-2185; Soutschek J. *et al.*, *Nature*, 2004, 432:173-178; Pardridge W.M., *Expert Opin. Biol. Ther.*, 2004, July, 4(7):1103-1113), each of which is incorporated herein by reference in its entirety.

10 In another embodiment, the decrease in NPR-A activity (e.g., a reduction in NPR-A expression) may be achieved by administering an analogue of ANP (e.g., ANP4-23) or non-peptide antagonists (e.g., HS-142-1; Rutherford *et al.*, *Br. J. Pharmacol.*, 1994, 113:931-939; El-Ayoubi *et al.*, *Br. J. Pharmacol.*, 2005, Feb. 07, Epub ahead of print; Delpont C. *et al.*, *Eur. J. Pharmacol.*, 1992, 224(2-3):183-188; Ohyama Y. *et al.*,
15 *Biochem. Biophys. Res. Commun.*, 1992, 189(1):336-342). In another embodiment, the agent is an anti-human NPR-A function-blocking antibody (preferably, humanized), or soluble NPR-A or NPR-C (as a receptor decoy). Other examples of agents include NPR-A antagonists that specifically inhibit cGMP-dependent protein kinase (PKG) such as A71915 and KT5823 (Pandey K.N. *et al.*, *Biochemical and Biophysical Research*
20 *Communications*, 2000, 271:374-379).

The methods of the invention may include further steps. In some embodiments, a subject with the relevant inflammatory disorder and/or cell proliferation disorder is identified or a patient at risk for the disorder is identified. A patient may be someone who has not been diagnosed with the disease or condition (diagnosis, prognosis, and/or
25 staging) or someone diagnosed with disease or condition (diagnosis, prognosis, monitoring, and/or staging), including someone treated for the disease or condition (prognosis, staging, and/or monitoring). Alternatively, the person may not have been diagnosed with the disease or condition but suspected of having the disease or condition based either on patient history or family history, or the exhibition or observation of
30 characteristic symptoms.

In one aspect, the therapeutic method of the invention involves administering a natriuretic hormone peptide (NP), or a fragment, homolog or variant thereof, or a nucleic acid sequence encoding an NP, or a fragment, homolog, or variant thereof, to a patient.

The present inventor has demonstrated that a prolonged, substantial reduction of tumor burden in lungs can be achieved by intranasal delivery of pDNA-encoding a peptide comprising amino acid residues 73 to 102 (NP73-102). Without being bound by theory, the NP decreased viability due to the induction of apoptosis in a lung adenocarcinoma cell line A549 cell, and can reduce tumorigenesis and metastasis in a number of cancers.

In specific embodiments, the peptides used in the subject invention comprise at least one amino acid sequence selected from the group consisting of NP₁₋₃₀, NP₃₁₋₆₇, NP₇₉₋₉₈, and NP₇₃₋₁₀₂, (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:5, respectively), SEQ ID NO:6, or a biologically active fragment or homolog thereof. In some embodiments, a combination of NP or NP-encoding nucleic acid sequences is utilized. In one embodiment, the peptide utilized does not consist of the amino acid sequence of NP₉₉₋₁₂₆ (SEQ ID NO: 4).

In another aspect, the therapeutic method of the invention involves administering an agent that reduces activity of the natriuretic peptide receptor-A (also known in the art as NPRA, NPR-A, and guanylate cyclase A) to a patient, wherein the agent is administered in an amount effective to reduce receptor (NPR-A) activity. NPR-A activity can be determined, for example, by one or more of the following biological parameters: production/accumulation of cGMP, expression of the NPR-A (transcription or translation), and/or cellular internalization of the NPR-A.

According to the gene therapy method of the present invention, the NP-encoding nucleic acid sequence is administered locally at the target site (*e.g.*, at the site of cancer or pre-cancer), or systemically to the patient. In order to treat cancer of the lung, for example, the NP-encoding nucleic acid sequence is preferably administered to the airways of the patient, *e.g.*, nose, sinus, throat and lung, for example, as nose drops, by nebulization, vaporization, or other methods known in the art. More preferably, the nucleic acid sequence encoding NP is administered to the patient orally or intranasally, or otherwise intratracheally. For example, the nucleic acid sequence can be inhaled by the patient through the oral or intranasal routes, or injected directly into tracheal or bronchial tissue.

In specific embodiments, the nucleic acid sequences used in the subject invention encode at least one amino acid sequence selected from the group consisting of NP₁₋₃₀, NP₃₁₋₆₇, NP₇₉₋₉₈, NP₉₉₋₁₂₆, and NP₇₃₋₁₀₂, (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3,

SEQ ID NO:4, and SEQ ID NO:5, respectively), SEQ ID NO:6, or a biologically active fragment or homolog of any of the foregoing.

Preferably, the nucleic acid sequence encoding the NP is administered with a nucleic acid sequence that is operatively linked with the NP-encoding nucleic acid sequence and operates as a regulatory sequence. For example, the regulatory sequence can be a promoter sequence that controls transcription and drives expression of the NP-encoding nucleic acid sequence at the desired site, such as at, or adjacent to, the patient's respiratory epithelial cells. The promoter can be a constitutive or inducible promoter to allow selective transcription. The promoter can be a vertebrate or viral promoter. 10 Optionally, enhancers may be used to obtain desired transcription levels. An enhancer is generally any non-translated nucleic acid sequence that works contiguously with the coding sequence (in *cis*) to change the basal transcription level dictated by the promoter.

The NP-encoding nucleic acid sequences used in the methods, expression vectors, and pharmaceutical compositions of the present invention are preferably isolated. 15 According to the present invention, an isolated nucleic acid molecule or nucleic acid sequence, is a nucleic acid molecule or sequence that has been removed from its natural milieu. As such, "isolated" does not necessarily reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule or sequence useful in the present composition can include DNA, RNA, or any derivatives of either DNA or 20 RNA. An isolated nucleic acid molecule or sequence can be double stranded (*i.e.*, containing both a coding strand and a complementary strand) or single stranded.

A nucleic acid molecule can be isolated from a natural source, or it can be produced using recombinant DNA technology (*e.g.*, polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Nucleic acid molecules can be generated 25 or modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of 30 a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the

sequence of nucleotides on the nucleic acid molecule, the two phrases are used interchangeably herein. As used herein, a "coding" nucleic acid sequence refers to a nucleic acid sequence that encodes at least a portion of a peptide or protein (e.g., a portion of an open reading frame), and can more particularly refer to a nucleic acid sequence encoding a peptide or protein which, when operatively linked to a transcription control sequence (e.g., a promoter sequence), can express the peptide or protein.

The nucleotide sequences encoding NP used in the subject invention include "homologous" or "modified" nucleotide sequences. Modified nucleic acid sequences will be understood to mean any nucleotide sequence obtained by mutagenesis according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the normal sequences. For example, mutations in the regulatory and/or promoter sequences for the expression of a polypeptide that result in a modification of the level of expression of a polypeptide according to the invention provide for a "modified nucleotide sequence". Likewise, substitutions, deletions, or additions of nucleic acids to the polynucleotides of the invention provide for "homologous" or "modified" nucleotide sequences. In various embodiments, "homologous" or "modified" nucleic acid sequences have substantially the same biological or serological activity as the native (naturally occurring) natriuretic peptide. A "homologous" or "modified" nucleotide sequence will also be understood to mean a splice variant of the polynucleotides of the instant invention or any nucleotide sequence encoding a "modified polypeptide" as defined below.

A homologous nucleotide sequence, for the purposes of the present invention, encompasses a nucleotide sequence having a percentage identity with the bases of the nucleotide sequences of between at least (or at least about) 20.00% to 99.99% (inclusive). The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire sequence length.

In various embodiments, homologous sequences exhibiting a percentage identity with the bases of the nucleotide sequences of the present invention can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94,

95, 96, 97, 98, or 99 percent identity with the polynucleotide sequences of the instant invention. Homologous nucleotide and amino acid sequences include mammalian homologs of the human NP sequences.

The NP homologs include peptides containing, as a primary amino acid sequence, all or part of an exemplified NP polypeptide sequence. The NP homologs thus include NP polypeptides having conservative substitutions, *i.e.*, altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is biologically active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. In one aspect of the present invention, conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs (see Table 1). Conservative substitutions also include substitutions by amino acids having chemically modified side chains which do not eliminate the biological activity of the resulting NP homolog.

Table 1.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman *Proc. Natl. Acad. Sci. USA*, 1988, 85(8):2444-2448; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Thompson *et al. Nucleic Acids Res.*, 1994, 22(2):4673-4680; Higgins *et al. Methods Enzymol.*, 1996,

266:383-402; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Altschul *et al. Nature Genetics*, 1993, 3:266-272).

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those
 5 described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; York (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; York (1993); Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G.,
 10 Academic Press, 1987; Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; York (1991); and Carillo *et al.*, SIAM J. Applied Math., 48:1073 (1988).

The methods, pharmaceutical compositions, and vectors of the present invention can utilize biologically active fragments of nucleic acid sequences encoding the 126-
 15 amino acid atrial natriuretic factor (ANF) prohormone, such as nucleic acid sequences encoding NP₁₋₃₀, NP₃₁₋₆₇, NP₇₉₋₉₈, NP₉₉₋₁₂₆, and NP₇₃₋₁₀₂, (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, respectively), SEQ ID NO:6, and including biologically active fragments of the nucleic acid sequences encoding SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

20 Representative fragments of the nucleotide sequences according to the invention will be understood to mean any polynucleotide fragment having at least 8 or 9 consecutive nucleotides, preferably at least 12 consecutive nucleotides, and still more preferably at least 15 or at least 20 consecutive nucleotides of the sequence from which it is derived, with retention of biological activity as described herein. The upper limit for
 25 such fragments is one nucleotide less than the total number of nucleotides found in the full-length sequence (or, in certain embodiments, of the full length open reading frame (ORF) identified herein).

In other embodiments, fragments can comprise consecutive nucleotides of 8, 9,
 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33,
 30 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121,

122, 123, 124, 125, 126, 127, and up to one nucleotide less than the full length ANF prohormone. In some embodiments, fragments comprise biologically active fragments of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

5 It is also well known in the art that restriction enzymes can be used to obtain biologically active fragments of the nucleic acid sequences, such as those encoding SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6. For example, *Bal31* exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for
10 example, Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.*, *J. Biol. Chem.*, 1983, 258:13006-13512.

The methods and pharmaceutical compositions of the present invention can utilize amino acid sequences that are biologically active fragments of the 126-amino acid atrial natriuretic factor (ANF) prohormone, such as NP₁₋₃₀, NP₃₁₋₆₇, NP₇₉₋₉₈, NP₉₉₋₁₂₆, and NP₇₃₋₁₀₂ (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5,
15 respectively), SEQ ID NO:6, and including biologically active fragments of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

Representative fragments of the polypeptides according to the invention will be understood to mean any polypeptide fragment having at least 8 or 9 consecutive amino
20 acids, preferably at least 12 amino acids, and still more preferably at least 15 or at least 20 consecutive amino acids of the polypeptide sequence from which it is derived, with retention of biological activity as described herein. The upper limit for such fragments is one amino acid less than the total number of amino acids found in the full-length sequence.

25 In other embodiments, fragments of the polypeptides can comprise consecutive amino acids of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99,
30 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and up to one amino acid less than the full-length ANF prohormone. Fragments of polypeptides can be any portion of the full-length ANF prohormone amino acid sequence (including human or non-human mammalian homologs

of the ANF prohormone) that exhibit biological activity as described herein, *e.g.*, a C-terminally or N-terminally truncated version of the ANF prohormone, or an intervening portion of the ANF prohormone. In some embodiments, fragments comprise biologically active fragments of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

The present invention can be practiced using other biologically equivalent forms of ANF fragments or homologs thereof as can be appreciated by the sequence comparison below. Sequence similarities between mouse and human forms of ANP are shown where areas of conservation are clearly seen.

NCBI BLAST Comparison of mouse (Query) to human (Sbjct) ANP a.a. sequences.

Query: 1 *MGSFSIT-*

LGFFLVLAFWLPGHIGANPVYSAVSNTDLMDFKNLLDHLEEKMPVEDEVMP

M SFS T + F L+LAF L G ANP+Y+AVSN

DLMDFKNLLDHLEEKMP+EDEV+PP

Sbjct: 1

MSSFSTTTVSFLLLLAFQLLGQTRANPMYNAVSNADLMDFKNLLDHLEEKMPLEDEVVPP

Query: 60

QALSEQTEEAGAALSSLPEVPPWTGEVNPPLRDGSALGRSPWDPSDXXXXXXXXXXXXXX

Q LSE EEAGAALS LPEVPPWTGEV+P RDG ALGR PWD SD

Sbjct: 61

QVLSEPNEEAGAALSPLPEVPPWTGEVSPAQRDGGALGRGPWDSSDRSALLKSKLRALLT

Query: 120 *GPRSLRRSSCFGGRIDRIGAQSGLGCNSFRY* 150

PRSLRRSSCFGGR+DRIGAQSGLGCNSFRY

Sbjct: 121 *APRSLRRSSCFGGRMDRIGAQSGLGCNSFRY* 151

The NP utilized in the subject invention can be peptide derivatives, such as those disclosed in U.S. Patent Publication No. 2004/0266673 (Bakis *et al.*), which is incorporated herein by reference in its entirety. These NP derivatives include an NP and a reactive entity coupled to the NP peptide. The reactive entity is able to covalently bond with a functionality on a blood component. Such NP derivatives are reported to have an extended half-life *in vivo*. The NP utilized in the subject invention can be a modified NP, such as those described in U.S. Patent Publication No. 2004/0002458 (Seilhamer *et al.*) and U.S. Patent Publication No. 2003/0204063 (Gravel *et al.*), which are incorporated herein by reference in their entirety.

The NP utilized may be a fusion polypeptide comprising an NP, or fragment or homolog thereof, and one or more additional polypeptides, such as another NP or a carrier protein, including those described in U.S. Patent Publication No. 2004/0138134 (Golembo *et al.*), which is incorporated herein by reference in its entirety. The NP utilized may be a chimeric polypeptide, such as those described in U.S. Patent Publication No. 2003/0069186 (Burnett *et al.*), which is incorporated herein by reference in its entirety. The fusion polypeptide or chimeric polypeptide may be administered to cells *in vitro* or *in vivo* directly (*i.e.*, as a polypeptide), or the fusion polypeptide may be administered as a polynucleotide encoding the fusion polypeptide with an operably linked promoter sequence. *See*, for example, Wang W. *et al.*, "Albubnp, a Recombinant B-type Natriuretic Peptide and Human Serum Albumin Fusion Hormone, as a Long-Term Therapy of Congestive Heart Failure", Pharmaceutical Research, Springer Science and Business Media B.V., Formerly Kluwer Academic Publishers B.V., ISSN:0724-8741, volume 21, Number 11, November, 2004, pages 2105-2111.

The NP includes all hydrates and salts of natriuretic peptides that can be prepared by those of skill in the art. Under conditions where the compounds of the present invention are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, alpha-ketoglutarate, and alpha-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts of NP may be obtained using standard procedures well known in the art, for example, by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The NP of the invention can be prepared by well-known synthetic procedures. For example, the polypeptides can be prepared by the well-known Merrifield solid support method. See Merrifield, *J. Amer. Chem. Soc.*, 1963, 85:2149-2154 and Merrifield (1965) *Science* 150:178-185. This procedure, using many of the same chemical reactions and blocking groups of classical peptide synthesis, provides a growing peptide chain anchored by its carboxyl terminus to a solid support, usually cross-linked polystyrene or styrene-divinylbenzene copolymer. This method conveniently simplifies the number of procedural manipulations since removal of the excess reagents at each step is effected simply by washing of the polymer.

Alternatively, these peptides can be prepared by use of well-known molecular biology procedures. Polynucleotides, such as DNA sequences, encoding the NP of the invention can be readily synthesized. Such polynucleotides are a further aspect of the present invention. These polynucleotides can be used to genetically engineer eukaryotic or prokaryotic cells, for example, bacteria cells, insect cells, algae cells, plant cells, mammalian cells, yeast cells or fungi cells for synthesis of the peptides of the invention.

For purposes of the present invention, the biological activity attributable to the homologs and fragments of NP and NP-encoding nucleic acid sequences means the capability to prevent or alleviate symptoms associated with inflammatory and/or cell proliferation disorders such as cancer. This biological activity can be mediated by one or more of the following mechanisms: increased production of intracellular Ca^{++} concentration (*e.g.*, in epithelial cells), increased production of nitric oxide (NO), and decreased activation of transcription factors such as NF κ B, ERK1,2 and/or AP1.

The methods of the subject invention also contemplate the administration of cells that have been genetically modified to produce NP, or biologically active fragments, variants, or homologs thereof. Such genetically modified cells can be administered alone or in combinations with different types of cells. Thus, genetically modified cells of the invention can be co-administered with other cells, which can include genetically modified

cells or non-genetically modified cells. Genetically modified cells may serve to support the survival and function of the co-administered cells, for example.

The term "genetic modification" as used herein refers to the stable or transient alteration of the genotype of a cell of the subject invention by intentional introduction of exogenous nucleic acids by any means known in the art (including for example, direct transmission of a polynucleotide sequence from a cell or virus particle, transmission of infective virus particles, and transmission by any known polynucleotide-bearing substance) resulting in a permanent or temporary alteration of genotype. The nucleic acids may be synthetic, or naturally derived, and may contain genes, portions of genes, or other useful polynucleotides in addition to those encoding NP. A translation initiation codon can be inserted as necessary, making methionine the first amino acid in the sequence. The term "genetic modification" is not intended to include naturally occurring alterations such as that which occurs through natural viral activity, natural genetic recombination, or the like. The genetic modification may confer the ability to produce NP, wherein the cell did not previously have the capability, or the modification may increase the amount of NP endogenously produced by the cell, *e.g.*, through increased expression.

Exogenous nucleic acids and/or vectors encoding NP can be introduced into a cell by viral vectors (retrovirus, modified herpes virus, herpes virus, adenovirus, adeno-associated virus, lentivirus, and the like) or direct DNA transfection (lipofection, chitosan-nanoparticle mediated transfection, calcium phosphate transfection, DEAE-dextran, electroporation, and the like), microinjection, cationic lipid-mediated transfection, transduction, scrape loading, ballistic introduction and infection (see, for example, Sambrook *et al.* [1989] *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Preferably, the exogenous polynucleotide encoding the NP is operably linked to a promoter sequence that permits expression of the polynucleotide in a desired tissue within the patient. The promoters can be inducible, tissue-specific, or event-specific, as necessary.

The genetically modified cell may be chosen from eukaryotic or prokaryotic systems, for example, bacterial cells (Gram negative or Gram positive), yeast cells, animal cells, plant cells, and/or insect cells using baculovirus vectors, for example. In

some embodiments, the genetically modified cell for expression of the nucleic acid sequences encoding NP, are human or non-human mammal cells.

According to the methods of the present invention, NP or polynucleotides encoding NP can be administered to a patient in order to alleviate (e.g., reduce or eliminate) a variety of symptoms associated with cancers, in various stages of pathological development. Treatment with NP or nucleic acid sequences encoding NP is intended to include prophylactic intervention to prevent or reduce cancer cell growth (e.g., tumor growth) and onset of the symptoms associated with cancer cell growth (e.g., tumor growth), such as pain. The nucleic acid sequences and pharmaceutical compositions of the invention can be co-administered (concurrently or consecutively) to a patient with other therapeutic agents useful for treating cancers of the lung, ovarian, breast, as well as melanomas.

Suitable expression vectors for NP include any that are known in the art or yet to be identified that will cause expression of NP-encoding nucleic acid sequences in mammalian cells. Suitable promoters and other regulatory sequences can be selected as is desirable for a particular application. The promoters can be inducible, tissue-specific, or event-specific, as necessary. For example, the cytomegalovirus (CMV) promoter (Boshart *et al.*, *Cell*, 1985, 41:521-530) and SV40 promoter (Subramani *et al.*, *Mol. Cell. Biol.*, 1981, 1:854-864) have been found to be suitable, but others can be used as well. Optionally, the NP-encoding nucleic acid sequences used in the subject invention include a sequence encoding a signal peptide upstream of the NP-encoding sequence, thereby permitting secretion of the NP from a host cell. Also, various promoters may be used to limit the expression of the peptide in specific cells or tissues, such as lung cells.

A tissue-specific and/or event-specific promoter or transcription element that responds to the target microenvironment and physiology can also be utilized for increased transgene expression at the desired site. There has been an immense amount of research activity directed at strategies for enhancing the transcriptional activity of weak tissue-specific promoters or otherwise increasing transgene expression with viral vectors. It is possible for such strategies to provide enhancement of gene expression equal to one or two orders of magnitude, for example (see Nettelbeck *et al.*, *Gene Ther.*, 1998, 5(12):1656-1664 and Qin *et al.*, *Hum. Gene Ther.*, 1997, 8(17):2019-2019, the abstracts of which are submitted herewith for the Examiner's convenience). Examples of cardiac-specific promoters are the ventricular form of MLC-2v promoter (see, Zhu *et al.*, *Mol.*

Cell Biol., 1993, 13:4432-4444, Navankasattusas *et al.*, *Mol. Cell Biol.*, 1992, 12:1469-1479, 1992) and myosin light chain-2 promoter (Franz *et al.*, *Circ. Res.*, 1993, 73:629-638). The E-cadherin promoter directs expression specific to epithelial cells (Behrens *et al.*, *PNAS*, 1991, 88:11495-11499), while the estrogen receptor (ER) 3 gene promoter
 5 directs expression specifically to the breast epithelium (Hopp *et al.*, *J. Mammary Gland Biol. Neoplasia*, 1998, 3:73-83). The human C-reactive protein (CRP) gene promoter (Ruther *et al.*, *Oncogene* 8:87-93, 1993) is a liver-specific promoter. An example of a muscle-specific gene promoter is human enolase (ENO3) (Peshavaria *et al.*, *Biochem. J.*, 1993, 292(Pt 3):701-704). A number of brain-specific promoters are available such as the
 10 thy-1 antigen and gamma-enolase promoters (Vibert *et al.*, *Eur. J. Biochem.* 181:33-39, 1989). The prostate-specific antigen promoter provides prostate tissue specificity (Pang *et al.*, *Gene Ther.*, 1995, 6(11):1417-1426; Lee *et al.*, *Anticancer Res.*, 1996, 16(4A):1805-1811). The surfactant protein B promoter provides lung specificity (Strayer *et al.*, *Am. J. Respir. Cell Mol. Biol.*, 1998, 18(1):1-11). Any of the aforementioned
 15 promoters may be selected for targeted or regulated expression of the NP-encoding polynucleotide.

Various viral or non-viral vectors may be used to deliver polynucleotides encoding NP to cells *in vitro* or *in vivo*, resulting in expression and production of NP. Tissue-specific promoters or event-specific promoters may be utilized with
 20 polynucleotides encoding NP to further optimize and localize expression at target sites, such as within diseased tissues (*e.g.*, cancer cells or tissues containing cancer cells). Robson *et al.* review various methodologies and vectors available for delivering and expressing a polynucleotide *in vivo* for the purpose of treating cancer (Robson, T. Hirst, D.G., *J. Biomed. and Biotechnol.*, 2003, 2003(2):110-137). Among the various targeting
 25 techniques available, transcriptional targeting using tissue-specific and event-specific transcriptional control elements is discussed. For example, Table 1 at page 112 of the Robson *et al.* publication lists several tissue-specific promoters useful in cancer therapy. Tables 2-4 of the Robson *et al.* publication list tumor-specific promoters, tumor environment-specific promoters, and exogenously controlled inducible promoters, many
 30 of which were available at the time the patent application was filed. The successful delivery and expression of the p53 tumor suppressor gene *in vivo* has been documented (Horowitz, J. *Curr. Opin. Mol. Ther.*, 1999, 1(4):500-509; Von Gruenigen, V.E. *et al. Int.*

J. Gynecol. Cancer, 1999, 9(5):365-372; Fujiwara, T. *et al.*, *Mol. Urol.*, 2000, 4(2):51-54, respectively).

Many techniques for delivery of drugs and proteins are available in the art to reduce the effects of enzymatic degradation, to facilitate cell uptake, and to reduce any potential toxicity to normal (undiseased) cells, *etc.* Such methods and reagents can be utilized for administration of NP to cells *in vitro* or *in vivo*. For example, peptides known as "cell penetrating peptides" (CPP) or "protein transduction domains" (PTD) have an ability to cross the cell membrane and enter the cell. PTDs can be linked to a cargo moiety such as a drug, peptide, or full-length protein, and can transport the moiety across the cell membrane. One well characterized PTD is the human immunodeficient virus (HIV)-1 Tat peptide (see, for example, Frankel *et al.*, U.S. Patent Nos. 5,804,604; 5,747,641; 6,674,980; 5,670,617; and 5,652,122; Fawell, S. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, 91:664-668). Peptides such as the homeodomain of *Drosophila* antennapedia (ANTp) and arginine-rich peptides display similar properties (Derossi, D. *et al.*, *J. Biol. Chem.*, 1994, 269:10444-10450; Derossi, D. *et al.*, *Trends Cell Biol.*, 1998, 8:84-87; Rojas, M. *et al.*, *Nat. Biotechnol.*, 1998, 16:370-375; Futaki, S. *et al.*, *J. Biol. Chem.*, 2001, 276:5836-5840). VP22, a tegument protein from Herpes simplex virus type 1 (HSV-1), also has the ability to transport proteins across a cell membrane (Elliot *et al.*, *Cell*, 1997, 88:223-233; Schwarze S.R. *et al.*, *Trends Pharmacol. Sci.*, 2000, 21:45-48). A common feature of these carriers is that they are highly basic and hydrophilic (Schwarze S.R. *et al.*, *Trends Cell Biol.*, 2000, 10:290-295). Coupling of these carriers to marker proteins such as beta-galactosidase has been shown to confer efficient internalization of the marker protein into cells. More recently, chimeric, in-frame fusion proteins containing these carriers have been used to deliver proteins to a wide spectrum of cell types both *in vitro* and *in vivo*. For example, VP22-p53 chimeric protein retained its ability to spread between cells and its pro-apoptotic activity, and had a widespread cytotoxic effect in p53 negative human osteosarcoma cells *in vitro* (Phelan, A. *et al.*, *Nature Biotechnol.*, 1998, 16:440-443). Intraperitoneal injection of the beta-galactosidase protein fused to the HIV-1 Tat peptide resulted in delivery of the biologically active fusion protein to all tissues in mice, including the brain (Schwarze S.R. *et al.*, *Science*, 1999, 285:1569-1572).

Liposomes of various compositions can also be used for site-specific delivery of proteins and drugs (Witschi, C. *et al.*, *Pharm. Res.*, 1999, 16:382-390; Yeh, M.K. *et al.*,

Pharm. Res., 1996, 1693-1698). The interaction between the liposomes and the protein cargo usually relies on hydrophobic interactions or charge attractions, particularly in the case of cationic lipid delivery systems (Zelphati, O. *et al.*, *J. Biol. Chem.*, 2001, 276:35103-35110). Tat peptide-bearing liposomes have also been constructed and used
5 to deliver cargo directly into the cytoplasm, bypassing the endocytotic pathway (Torchilin V.P. *et al.*, *Biochim. Biophys. Acta-Biomembranes*, 2001, 1511:397-411; Torchilin V.P. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2001, 98:8786-8791). When encapsulated in sugar-grafted liposomes, pentamidine isethionate and a derivative have been found to be more potent in comparison to normal liposome-encapsulated drug or to the free drug (Banerjee,
10 G. *et al.*, *J. Antimicrob. Chemother.*, 1996, 38(1):145-150). A thermo-sensitive liposomal taxol formulation (heat-mediated targeted drug delivery) has been administered *in vivo* to tumor-bearing mice in combination with local hyperthermia, and a significant reduction in tumor volume and an increase in survival time was observed compared to the equivalent dose of free taxol with or without hyperthermia (Sharma, D. *et al.*, *Melanoma*
15 *Res.*, 1998, 8(3):240-244). Topical application of liposome preparations for delivery of insulin, IFN-alpha, IFN-gamma, and prostaglandin E1 have met with some success (Cevc G. *et al.*, *Biochim. Biophys. Acta*, 1998, 1368:201-215; Foldvari M. *et al.*, *J. Liposome Res.*, 1997, 7:115-126; Short S.M. *et al.*, *Pharm. Res.*, 1996, 13:1020-1027; Foldvari M. *et al.*, *Urology*, 1998, 52(5):838-843; U.S. Patent No. 5,853,755).

20 Antibodies represent another targeting device that may make liposome uptake tissue-specific or cell-specific (Mastrobattista, E. *et al.*, *Biochim. Biophys. Acta*, 1999, 1419(2):353-363; Mastrobattista, E. *et al.*, *Adv. Drug Deliv. Rev.*, 1999, 40(1-2):103-127). The liposome approach offers several advantages, including the ability to slowly release encapsulated drugs and proteins, the capability of evading the immune system and
25 proteolytic enzymes, and the ability to target tumors and cause preferentially accumulation in tumor tissues and their metastases by extravasation through their leaky neovasculature. Other carriers have also been used to deliver anti-cancer drugs to neoplastic cells, such as polyvinylpyrrolidone nanoparticles and maleylated bovine serum albumin (Sharma, D. *et al.*, *Oncol. Res.*, 1996, 8(7-8):281-286; Mukhopadhyay, A. *et al.*,
30 *FEBS Lett.*, 1995, 376(1-2):95-98). Thus, using targeting and encapsulation technologies, which are very versatile and amenable to rational design and modification, delivery of NP to desired cells can be facilitated. Furthermore, because many liposome compositions

are also viable delivery vehicles for genetic material, many of the advantages of liposomes are equally applicable to polynucleotides encoding NP.

As indicated above, the pharmaceutical composition of the present invention can include a liposome component. According to the present invention, a liposome comprises
5 a lipid composition that is capable of fusing with the plasma membrane of a cell, thereby allowing the liposome to deliver a nucleic acid molecule and/or a protein composition into a cell. Some preferred liposomes include those liposomes commonly used in gene delivery methods known to those of skill in the art. Some preferred liposome delivery vehicles comprise multilamellar vesicle (MLV) lipids and extruded lipids, although the
10 invention is not limited to such liposomes. Methods for preparation of MLVs are well known in the art. "Extruded lipids" are also contemplated. Extruded lipids are lipids that are prepared similarly to MLV lipids, but which are subsequently extruded through filters of decreasing size, as described in Templeton *et al.*, *Nature Biotech.*, 1997, 15:647-652, which is incorporated herein by reference in its entirety. Small unilamellar vesicle (SUV)
15 lipids can also be used in the compositions and methods of the present invention. Other preferred liposome delivery vehicles comprise liposomes having a polycationic lipid composition (*i.e.*, cationic liposomes). For example, cationic liposome compositions include, but are not limited to, any cationic liposome complexed with cholesterol, and without limitation, include DOTMA and cholesterol, DOTAP and cholesterol, DOTIM
20 and cholesterol, and DDAB and cholesterol. Liposomes utilized in the present invention can be any size, including from about 10 to 1000 nanometers (nm), or any size in between.

A liposome delivery vehicle can be modified to target a particular site in a mammal, thereby targeting and making use of an NP-encoding nucleic acid molecule of
25 the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle. Manipulating the chemical formula of the lipid portion of the delivery vehicle can elicit the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome
30 so that the liposome fuses with particular cells having particular charge characteristics. In one embodiment, other targeting mechanisms, such as targeting by addition of exogenous targeting molecules to a liposome (*i.e.*, antibodies) may not be a necessary component of the liposome of the present invention, since effective immune activation at

immunologically active organs can already be provided by the composition when the route of delivery is intravenous or intraperitoneal, without the aid of additional targeting mechanisms. However, in some embodiments, a liposome can be directed to a particular target cell or tissue by using a targeting agent, such as an antibody, soluble receptor or ligand, incorporated with the liposome, to target a particular cell or tissue to which the targeting molecule can bind. Targeting liposomes are described, for example, in Ho *et al.*, *Biochemistry*, 1986, 25: 5500-6; Ho *et al.*, *J Biol Chem*, 1987a, 262: 13979-84; Ho *et al.*, *J Biol Chem*, 1987b, 262: 13973-8; and U.S. Patent No. 4,957,735 to Huang *et al.*, each of which is incorporated herein by reference in its entirety). In one embodiment, if avoidance of the efficient uptake of injected liposomes by reticuloendothelial system cells due to opsonization of liposomes by plasma proteins or other factors is desired, hydrophilic lipids, such as gangliosides (Allen *et al.*, *FEBS Lett*, 1987, 223: 42-6) or polyethylene glycol (PEG)-derived lipids (Klibanov *et al.*, *FEBS Lett*, 1990, 268: 235-7), can be incorporated into the bilayer of a conventional liposome to form the so-called sterically-stabilized or "stealth" liposomes (Woodle *et al.*, *Biochim Biophys Acta*, 1992, 1113: 171-99). Variations of such liposomes are described, for example, in U.S. Patent No. 5,705,187 to Unger *et al.*, U.S. Patent No. 5,820,873 to Choi *et al.*, U.S. Patent No. 5,817,856 to Tirosh *et al.*; U.S. Patent No. 5,686,101 to Tagawa *et al.*; U.S. Patent No. 5,043,164 to Huang *et al.*, and U.S. Patent No. 5,013,556 to Woodle *et al.*, all of which are incorporated herein by reference in their entireties).

The NP-encoding nucleic acid sequences of the present invention can be conjugated with chitosan. For example, DNA chitosan nanospheres can be generated, as described by Roy, K. *et al.* (1999, *Nat Med* 5:387). Chitosan allows increased bioavailability of the NP-encoding nucleic acid sequences because of protection from degradation by serum nucleases in the matrix and thus has great potential as a mucosal gene delivery system. Chitosan also has many beneficial effects, including anticoagulant activity, wound-healing properties, and immunostimulatory activity, and is capable of modulating immunity of the mucosa and bronchus-associated lymphoid tissue.

Mammalian species which benefit from the disclosed methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos,

such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales. The terms "patient" and "subject" are used interchangeably herein
5 are intended to include such human and non-human mammalian species. According to the method of the present invention, human or non-human mammalian NP (or nucleic acid sequences encoding human or non-human mammalian NP) can be administered to the patient. The NP may be naturally occurring within the patient's species or a different mammalian species. The expression vectors used in the subject invention can comprise
10 nucleic acid sequences encoding any human or non-human mammalian NP. In instances where genetically modified cells are administered to a patient, the cells may be autogenic, allogeneic, or xenogeneic, for example.

In another aspect, the present invention concerns pharmaceutical compositions containing a therapeutically effective amount of agent that reduces NPR-A activity, such
15 as an NP, or polynucleotides encoding NP, and a pharmaceutically acceptable carrier. Preferably, if the agent is a polynucleotide, such as an NP-encoding nucleic acid sequence, the polynucleotide is contained within an expression vector, such as plasmid DNA or a virus. Pharmaceutical compositions including a therapeutically effective amount of an agent that reduces NPR-A activity such as NP, or nucleic acid sequences
20 encoding NP, and a pharmaceutically acceptable carrier, can be administered to a patient by any effective route, including local or systemic delivery. Administration can be continuous or at distinct intervals as can be determined by a person skilled in the art.

The agent that reduces NPR-A activity, such as NP or polynucleotides encoding NP (and pharmaceutical compositions containing them), can be administered to a patient
25 by any route that results in prevention (or reduction of onset) or alleviation of symptoms associated with cancer, such as pain. For example, the agent (*e.g.*, NP or NP-encoding nucleic acid molecule) can be administered parenterally, intravenously (I.V.), intramuscularly (I.M.), subcutaneously (S.C.), intradermally (I.D.), topically, transdermally, orally, intranasally, *etc.*

30 If desired, the pharmaceutical composition can be adapted for administration to the airways of the patient, *e.g.*, nose, sinus, throat and lung, for example, as nose drops, as nasal drops, by nebulization as an inhalant, vaporization, or other methods known in the art. Examples of intranasal administration can be by means of a spray, drops, powder or

gel and also described in US Patent No. 6,489,306, which is incorporated herein by reference in its entirety. One embodiment of the present invention is the administration of the invention as a nasal spray. Alternate embodiments include administration through any oral or mucosal routes, sublingual administration and even eye drops. However,
5 other means of drug administrations are well within the scope of the present invention.

The pharmaceutical compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier" includes any of the standard pharmaceutically acceptable carriers. The pharmaceutically
10 acceptable carrier can include diluents, adjuvants, and vehicles, as well as implant carriers, and inert, non-toxic solid or liquid fillers, diluents, or encapsulating material that does not react with the active ingredients of the invention. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions. The carrier can be a solvent or dispersing medium containing, for
15 example, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. Formulations containing pharmaceutically acceptable carriers are described in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Sciences* (Martin E.W., 1995, Easton Pennsylvania, Mack
20 Publishing Company, 19th ed.), which is incorporated herein by reference in its entirety, describes formulations that can be used in connection with the subject invention.

Pharmaceutical compositions of the present invention useful for parenteral injection can include pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for
25 reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for
30 example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for parenteral administration include, for example, aqueous injectable solutions that may contain antioxidants, buffers, and solutes which render the

formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that, in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

The pharmaceutical compositions used in the methods of the present invention may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption, such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the active agent (*e.g.*, NP), it is desirable to slow the absorption from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the NP or NP-encoding polynucleotide then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered NP or NP-encoding polynucleotide is accomplished by dissolving or suspending the NP in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the agent (*e.g.*, NP or NP-encoding polynucleotide) in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of active agent (*e.g.*, NP or NP-encoding polynucleotide) to polymer and the nature of the particular polymer employed, the rate of release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared

by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agents (NP or NP-encoding polynucleotide) are mixed with at least one pharmaceutically acceptable excipient or carrier such as sodium nitrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay; and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hardfilled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. Optionally, the solid dosage forms contain opacifying agents, and can be of a composition that releases the NP or NP-encoding polynucleotide only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

The active agents (NP or NP-encoding polynucleotide) can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the NP or NP-encoding polynucleotide, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder, which may be pressurized or non-pressurized. In nonpressurized powder compositions, the active ingredients in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 μm in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 μm .

Alternatively, the pharmaceutical composition may be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium or the entire composition is preferably such that the active ingredients do not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent. The surface active agent may be a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

The compositions and methods of the invention can further incorporate permeation enhancers, such as those described in U.S. Patent Publication No. 2003/0147943 (Luo *et al.*), penetrating peptides capable of facilitating penetration of an NP, or an NP-encoding polynucleotide, across a biological barrier, such as those described in U.S. Patent Publication No. 2004/0146549 (Ben-Sasson *et al.*), enhancer compounds that enhance the absorption of a polypeptide in the respiratory tract, such as those described in U.S. Patent Publication No. 2004/0171550 (Backstrom *et al.*), and suspension vehicles, such as those described in U.S. Patent Publication No. 2004/0224903 (Berry *et al.*), each of which are incorporated herein by reference in their entirety.

The agent that reduces NPR-A activity (such as NP or NP-encoding polynucleotide) is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight, and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. For example, an effective amount of NP-encoding polynucleotide is that amount necessary to provide an effective amount of NP, when expressed *in vivo* or *in vitro*. The amount of the agent (*e.g.*, NP or NP-encoding nucleic acid molecule) must be effective to achieve some improvement including, but not limited to, improved survival rate, more rapid recovery, total prevention of symptoms associated with an inflammatory or cell proliferation disorder, such as cancer, or improvement or elimination of symptoms associated with an inflammatory or cell proliferation disorder, such as cancer, and other indicators as are selected as appropriate measures by those skilled in the art. In accordance with the present invention, a suitable single dose size is a dose that is capable of preventing or alleviating (reducing or eliminating) a symptom in a patient when administered one or more times over a suitable time period. One of skill in the art can readily determine appropriate single dose sizes for local or systemic administration based on the size of a mammal and the route of administration.

In accordance with the invention, a mammal (such as a human) that is predisposed to or suffering from a physical disorder may be treated by administering to the mammal an effective amount of an agent that reduces NPR-A activity (such as NP or NP-encoding polynucleotide), in combination with a pharmaceutically acceptable carrier or excipient

therefore (as described below). Physical disorders treatable with the compositions and methods of the present invention include any physical disorder that may be delayed, prevented cured or otherwise treated by administration of an agent that reduces NPR-A activity (such as NP or NP-encoding polynucleotide) in a mammal suffering from or
5 predisposed to the physical disorder. Such physical disorders include, but are not limited to, a variety of carcinomas and other cancers, such as skin cancers (including melanomas and Kaposi's Sarcoma), oral cavity cancers, lung cancers, breast cancers, prostatic cancers, bladder cancers, liver cancers, pancreatic cancers, cervical cancers, ovarian cancers, head and neck cancers, colon cancers, germ cell cancers (including
10 teratocarcinomas) and leukemias. Other physical disorders treatable by the methods of the present invention include inflammatory disorders such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, pelvic inflammatory disease, and Crohn's disease. The methods of the invention may also be used to treat a mammal suffering from or predisposed to a fibrotic disorder, including pulmonary fibrosis, cystic
15 fibrosis, endomyocardial fibrosis, hepatic fibrosis (particularly hepatic cirrhosis), myelofibrosis, scleroderma, and systemic sclerosis. Other physical disorders treatable by the methods of the invention include osteoporosis, atherosclerosis, and ocular disorders such as corneal ulceration and diabetic retinopathy. The methods of the present invention may also be used in the prevention of disease progression, such as in chemoprevention of
20 the progression of a premalignant lesion to a malignant lesion, and to treat a mammal suffering from, or predisposed to, other physical disorders that respond to treatment with compositions that differentially modulate gene expression.

Cell proliferation disorders include but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary
25 tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases. Those disorders also include lymphomas, sarcomas, and leukemias.

Cancers of any organ can be treated, including cancers of, but are not limited to, *e.g.*, colon, pancreas, breast, prostate, bone, liver, kidney, lung, testes, skin, pancreas, stomach, colorectal cancer, renal cell carcinoma, hepatocellular carcinoma, melanoma,
30 *etc.*

Examples of breast cancer include, but are not limited to, invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma *in situ*, and lobular carcinoma *in situ*. Examples of cancers of the respiratory tract include, but are not limited to, small-

cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma. Examples of brain cancers include, but are not limited to, brain stem and hypophthalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor. Tumors of the male reproductive organs include, but are not limited to, prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to, endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus. Tumors of the digestive tract include, but are not limited to, anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers. Tumors of the urinary tract include, but are not limited to, bladder, penile, kidney, renal pelvis, ureter, and urethral cancers. Eye cancers include, but are not limited to, intraocular melanoma and retinoblastoma. Examples of liver cancers include, but are not limited to, hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma. Skin cancers include, but are not limited to, squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer. Head-and-neck cancers include, but are not limited to, laryngeal, hypopharyngeal, nasopharyngeal, and/or oropharyngeal cancers, and lip and oral cavity cancer. Lymphomas include, but are not limited to, AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system. Sarcomas include, but are not limited to, sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma. Leukemias include, but are not limited to, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia. In addition to reducing the proliferation of tumor cells, agents that reduce NPR-A activity can also cause tumor regression, *e.g.*, a decrease in the size of a tumor, or in the extent of cancer in the body.

In addition to chemotherapeutic agents, the methods and compositions of the subject invention can incorporate treatments and agents utilizing, for example, angiogenesis inhibitors (Thalidomide, Bevacizumab), Bcl-2 antisense oligonucleotides (G3139), a PSA based vaccine, a PDGF receptor inhibitor (Gleevec), microtubule stabilizers (Epothilones), and a pro-apoptotic agent (Perifosine). Thus, an NP or NP-encoding polynucleotide can be administered to a patient in combination (simultaneously

or consecutively) with other agents for useful for treating inflammatory disorders and/or cell proliferation disorders. Likewise, the pharmaceutical compositions of the subject invention can include such agents.

The term "gene therapy", as used herein, refers to the transfer of genetic material (a polynucleotide, *e.g.*, DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (*e.g.*, a protein, polypeptide, peptide, or functional RNA) whose production *in vivo* is desired, such as NP. In addition to one or more NP, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic and/or diagnostic value. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy, cells are removed from a patient and, while being cultured, are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, *etc.*) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to produce the transfected gene product *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject, rather the gene to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. Alternatively, if the host gene is defective, the gene is repaired *in situ*. Thus, these genetically altered cells produce the transfected gene product (*e.g.*, NP) *in situ*.

The gene expression vector is capable of delivery/transfer of heterologous nucleic acid sequences (*e.g.*, NP-encoding nucleic acid sequences) into a host cell. The expression vector may include elements to control targeting, expression and transcription of the nucleic acid sequence in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle.

The expression vector can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. The expression vector can also include a selection gene.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang *et al.*, Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega *et al.*, Gene Targeting, CRC Press, Ann Arbor, Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and include, for example, stable or transient transfection, lipofection, electroporation, and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of a DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features

that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

In another aspect, the present invention concerns an isolated peptide comprising the amino acid sequence NP₇₃₋₁₀₂ (SEQ ID NO:5), or a biologically active fragment or homolog thereof. NP₇₃₋₁₀₂ is amino acids 73-102 of the 151-amino acid long human atrial natriuretic factor (ANF). In another aspect, the present invention concerns an isolated peptide comprising the amino acid sequence of SEQ ID NO:6, or a biologically active fragment or homolog thereof. SEQ ID NO:6 is a biologically active fragment of the human ANF. In another aspect, the present invention concerns an isolated nucleic acid molecule encoding the amino acid sequence of NP₇₃₋₁₀₂ (SEQ ID NO:5), or a biologically active fragment or homolog thereof. In another aspect, the present invention concerns an isolated nucleic acid molecule (SEQ ID NO:13) encoding the amino acid sequence of SEQ ID NO:6, or a biologically active fragment or homolog thereof.

As used herein, the terms "peptide", "polypeptide", and "protein" refer to amino acid sequences of any length unless otherwise specified.

Assays for Identifying Agents that Reduce Natriuretic Peptide Receptor-A Activity

The present invention also includes methods for identifying agents that reduce the activity of natriuretic peptide receptor-A (also known in the art as NPRA, NPR-A, and guanylate cyclase A) *in vitro* or *in vivo* (also referred to herein as the diagnostic method or screening assay of the invention). Such agents are potentially useful for treating inflammatory or cell proliferation disorders in a patient. In the therapeutic methods and assays of the invention, agents that reduce NPR-A activity include those that, for

example, reduce ANP-NPR-A induced c-GMP production, reduce expression of NPR-A, reduce cellular internalization of NPR-A, reduce recycling of NPR-A to the cell membrane, or otherwise interfere with the activity of the receptor.

Production of ANP-NPR-A induced cGMP production can be assayed and used as
5 a high-throughput method for screening agents for anti-proliferative (*e.g.*, anti-cancer) and anti-inflammatory activity. This assay can be carried out using a cell line that transiently or stably expresses the receptor for ANP, NPR-A (Pandey *et al.*, *J Biol Chem.* 2002, 277:4618- 4627) and libraries of agents, such as peptide and compound libraries, which can be novel or obtained commercially. An assay for cGMP can be performed to
10 select agents that are inhibitors of cGMP. Alternatively, ANP peptide can be linked with a moiety that can antagonize cGMP following internalization, which can be checked using a transiently or stably transfected cell line expressing NPR-A.

In the context of the screening assay of the invention, the terms "recombinant host cells", "host cells", "genetically modified host cells" "cells", "cell lines", "cell cultures",
15 and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, immaterial of the method by which the DNA is introduced into the cell or the subsequent disposition of the cell. The terms include the progeny of the original cell that has been transfected. Cells in primary culture can also be
20 used as recipients. Host cells can range in plasticity and proliferation potential. Host cells can be differentiated cells, progenitor cells, or stem cells, for example.

Host cells can be genetically modified with vectors to express (*e.g.*, overexpress) the NPR-A receptor, or a mutant, isoform, or other variant thereof, which may be a cloning vector or an expression vector, for example. The vector may be in the form of a
25 plasmid, a virus, (*e.g.*, a retrovirus or other virus), a viral particle, a phage, *etc.* The genetically modified host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants/transfectants or amplifying the receptor-encoding polynucleotide.

In one embodiment, the host cell is a human cell. In another embodiment, the host
30 cell is a non-human mammalian cell. Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences (*e.g.*, promoter sequences) that are compatible with the designated host are used. For example, among prokaryotic hosts, *Escherichia coli* may be used. Also, for example,

expression control sequences for prokaryotes include but are not limited to promoters, optionally containing operator portions, and ribosome binding sites. Eukaryotic hosts include yeast and mammalian cells in culture systems. *Pichia pastoris*, *Saccharomyces cerevisiae* and *S. carlsbergensis* are commonly used yeast hosts. Yeast-compatible
5 vectors carry markers that permit selection of successful transformants by conferring protrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2- μ origin of replication (Broach *et al. Meth. Enzymol.* 101:307, 1983), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences that will result in incorporation of an appropriate
10 fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include but are not limited to promoters for the synthesis of glycolytic enzymes, including the promoter for 3-phosphoglycerate kinase. (See, for example, Hess *et al. J. Adv. Enzyme Reg.* 7:149, 1968; Holland *et al. Biochemistry* 17:4900, 1978; and Hitzeman *J. Biol. Chem.* 255:2073, 1980). For example, some useful control systems are those that
15 comprise the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and, if secretion is desired, leader sequences from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type
20 organism.

Host cells useful for expression of polynucleotides encoding the NPR-A receptor may be primary cells or cells of cell lines. The host cells may be tumor cells (transformed cells) or non-tumor cells. Mammalian cell lines available as hosts for expression are known in the art and are available from depositories such as the American Type Culture
25 Collection. These include but are not limited to HeLa cells, human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and others.

The number of host cells used in a particular assay will vary with the objectives of the assay, the solid support used to support or contain the cell(s), if one is utilized *etc.*
30 Thus, in some protocols, the host cell may be a single cell. In other protocols, a plurality of host cells will be used.

In accordance with the screening assay of the invention, the polynucleotide encoding the NPR-A is operably linked to a promoter sequence. Suitable promoters

sequences for mammalian cells also are known in the art and include viral promoters such as that from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), bovine papilloma virus (BPV) and cytomegalovirus (CMV). Mammalian cells also may require terminator sequences and poly A addition sequences; enhancer sequences which
5 increase expression also may be included, and sequences which cause amplification of the gene also may be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which ensure integration of the appropriate sequences including the NPR-A receptor into the host genome. An example of such a mammalian expression system is described in
10 Gopalakrishnan *et al. Eur. J. Pharmacol.-Mol. Pharmacol.* 290: 237-246, 1995).

Candidate agents (and treatments) that may be tested by the screening assays of the present invention include polypeptides, non-peptide small molecules, biological agents, and any other source of candidate agents potentially having the ability to modulate (e.g., reduce) NPR-A activity. Candidate agents and treatments may be useful for the
15 treatment of inflammatory and/or cell proliferation disorders, such as cancer. Candidate agents can be virtually any substance and can encompass numerous chemical classes, including organic compounds or inorganic compounds. A candidate agent may be a substance such as genetic material, protein, lipid, carbohydrate, small molecules, a combination of any of two or more of foregoing, or other compositions. Candidate agents
20 may be naturally occurring or synthetic, and may be a single substance or a mixture. Candidate agents can be obtained from a wide variety of sources including libraries of compounds. A candidate agent can be or include, for example, a polypeptide, peptidomimetic, amino acid(s), amino acid analog(s), polynucleotide(s), polynucleotide analog(s), nucleotide(s), nucleotide analog(s), or other small molecule(s). A
25 polynucleotide may encode a polypeptide that potentially reduces NPR-A activity within the cell, or the polynucleotide may be a short interfering RNA (siRNA), a hairpin RNA (shRNA), antisense oligonucleotide, ribozyme, or other polynucleotide that targets an endogenous or exogenous gene for silencing of gene expression and potentially NPR-A activity within the cell. Candidate treatments may include exposure of the host cells to
30 any conditions that potentially reduce NPR-A activity within the host cells. The treatment may involve exposing the cells to an energy source, for example.

According to the screening assay of the invention, the method for identifying agents (which is intended to be inclusive of treatments) that reduce NPR-A activity is

used to identify an agent that is therapeutic for treating an inflammation disorder and/or cell proliferation disorder, such as cancer. In aspect, the screening assay comprising contacting a host cell with a candidate agent, wherein the host cell expresses NPR-A, or an active fragment or variant thereof; and determining whether activity of the receptor is reduced, wherein a decrease in receptor activity is indicative of a potentially therapeutic agent. The method can optionally include an additional step of comparing NPR-A activity in the presence of the candidate agent, with NPR-A activity in the absence of the candidate agent (e.g., or other positive or negative control). The determination of NPR-A activity may be quantitative, semi-quantitative, or qualitative.

Known methods for overexpressing NPR-A in host cells and determining intracellular cGMP may be utilized to determine whether NPR-A activity is reduced (Kumar *et al.*, *Hypertension*, 1997, 29(part 2):414-421; Khurana M.L. and Pandey K.N., *Endocrinology*, 1993, 133:2141-2149; Delport C. *et al.*, *Eur. J. Pharmacol.*, 1992, 224(2-3):183-188; Ohyama Y. *et al.*, *Biochem. Biophys. Res. Commun.*, 1992, 189(1):336-342; Sharma G.D. *et al.*, Expression of Atrial Natriuretic Peptide Receptor-A Antagonizes the Mitogen-Activated Protein Kinases (erk2 and P38^{MAPK}) in cultured human vascular Smooth Muscle Cells", in *Molecular and Cellular Biochemistry*, Springer Science + Business Media B.V., ISSN:0300-8177, Vol. 233, Number. 1-2, April 2002, pages 165-173; Pandey K.N. *et al.*, *Biochem. Biophys. Res. Commun.*, 2000, 271(2):374-379; Fujiseki Y. *et al.*, *Jpn. J. Pharmacol.*, 1999, 79(3):359-368; Pandey K.N., *Can. J. Physiol. Pharmacol.*, 2001, 79(8):631-639; Pandey K.N., *Mol. Cell. Biochem.*, 2002, 230(1-2):61-72; Sekiguchi T. *et al.*, *Gene*, 2001, 273:251-257; Chen S. *et al.*, *J. Am. Soc. Nephrol.*, 2005, 16:329-339; Pandey K.N. *et al.*, *J. Biol. Chem.*, 2002, 277(7):4618-4627; Pandey K.N. *et al.*, *Biochem. J.*, 2004, Dec. 1, Epub ahead of print; Roueau N. *et al.*, Poster #P10144, "Development of a Non-radioactive Homogenous HTS Platform to Measure the Activity of Guanylate Cyclase", Presented at 10th Annual SBS Conference and Exhibition, Orlando, FL, September 11-15, 2004, PERKINELMER BIOSIGNAL Inc., (Canada) each of which is incorporated herein by reference in its entirety). Functional truncations of NPR-A may also be used in the method of the invention (Pandey K.N. *et al.*, *Molecular Pharmacology*, 2000, 57:259-267, which is incorporated herein by reference in its entirety). For example, using the AlphaScreen, a very sensitive assay platform capable of detecting fmol levels of non-acetylated cGMP has been developed (Rouveau *et al.*, 2004). A biotinylated derivative of cGMP can be used as a tracer in a

competitive immunoassay format involving rabbit anti cGMP antibodies. The AlphaScreen signal is generated when streptavidin coated Donor beads and protein A coated Acceptor beads are brought into proximity by the formation of the biotin-cGMP / anti-cGMP IgG complex. Production of cGMP by either particulate or soluble forms of guanylate cyclase leads to a decrease of the AlphaScreen signal by inhibiting the formation of the biotin-cGMP / anti-cGMP IgG complex. Using this assay, the activity of the atrial natriuretic peptide receptor (NPR-A, particulate guanylate cyclase) overexpressed in CHO cells has been characterized as well as that of soluble guanylate cyclase. Pharmacological parameters and Z' values obtained indicate that the assay platform is amenable to HTS.

In addition to determining whether an agent reduces NPR-A activity *in vitro* (e.g., in a cellular or acellular assay) and/or *in vivo* (in a human or non-human patient, or an animal model), the method may further comprise determining whether the agent reduces the physiological effects or symptoms associated with an inflammatory disorder and/or cell proliferation disorder, such as cancer, *in vitro* and/or *in vivo* (e.g., in an animal model). For example, the method may further comprise determining whether the agent has an apoptotic effect on cancer cells *in vitro*. These steps may be carried out before, during, or after NPR-A activity is assayed.

Contacting steps in the assays (methods) of the invention can involve combining or mixing the candidate agent and the cell in a suitable receptacle, such as a reaction vessel, microvessel, tube, microtube, well, or other solid support. Host cells and/or candidate agents may be arrayed on a solid support, such as a multi-well plate. "Arraying" refers to the act of organizing or arranging members of a library, or other collection, into a logical or physical array. Thus, an "array" refers to a physical or logical arrangement of, e.g., library members (candidate agent libraries). A physical array can be any "spatial format" or physically gridded format" in which physical manifestations of corresponding library members are arranged in an ordered manner, lending itself to combinatorial screening. For example, samples corresponding to individual or pooled members of a candidate agent library can be arranged in a series of numbered rows and columns, e.g., on a multiwell plate. Similarly, host cells can be plated or otherwise deposited in microtitered, e.g., 96-well, 384-well, or-1536 well, plates (or trays). Optionally, host cells may be immobilized on the solid support.

A "solid support" (also referred to herein as a "solid substrate") has a fixed organizational support matrix that preferably functions as an organization matrix, such as a microtiter tray. Solid support materials include, but are not limited to, glass, polacryloylmorpholide, silica, controlled pore glass (CPG), polystyrene, polystyrene/latex, polyethylene, polyamide, carboxyl modified teflon, nylon and nitrocellulose and metals and alloys such as gold, platinum and palladium. The solid support can be biological, non-biological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.*, depending upon the particular application. Other suitable solid substrate materials will be readily apparent to those of skill in the art. The surface of the solid substrate may contain reactive groups, such as carboxyl, amino, hydroxyl, thiol, or the like for the attachment of nucleic acids, proteins, *etc.* Surfaces on the solid substrate will sometimes, though not always, be composed of the same material as the substrate. Thus, the surface can be composed of any of a wide variety of materials, for example, polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, membranes, or any of the above-listed substrate materials.

Measurement of NPR-A gene expression can be carried out using R.T-PCR, for example. Screening of candidate agents or treatments (*e.g.*, determination of NPR-A receptor activity) can be performed in a high-throughput format using combinatorial libraries, expression libraries, and the like. Other assays can be carried out on the host cells before, during, and/or after detection of NPR-A activity, and any or all assays may be carried out in an automated fashion, in a high-throughput format.

Alternatively, the aforementioned methods can be modified through the use of a cell-free assay. For example, instead of determining whether NPR-A activity in host cells is reduced by a candidate agent, extracts from host cells may be utilized and a fluorochrome or other detectable moiety can be associated with a nanoparticle or bead.

Once an agent has been determined to be one which reduces NPR-A activity, the agent can be combined with a pharmaceutically acceptable carrier. The method may further include a step of manufacturing the agent. The method may further include the step of packaging the agent.

Various methods of the present invention can include a step that involves comparing a value, level, feature, characteristic, property, *etc.* to a "suitable control",

referred to interchangeably herein as an "appropriate control". A "suitable control" or "appropriate control" is any control or standard familiar to one of ordinary skill in the art useful for comparison purposes. In one embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, *etc.* determined before, during, or after contacting an NPR-A receptor with a candidate agent, as described herein. For example, a transcription rate, mRNA level, translation rate, protein level, biological activity, cellular characteristic or property, genotype, phenotype, *etc.* can be determined prior to introducing a candidate into a cell or organism. In another embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, *etc.* determined in a cell or organism, *e.g.*, a control or normal cell or organism, exhibiting, for example, normal traits. In yet another embodiment, a "suitable control" or "appropriate control" is a predefined value, level, feature, characteristic, property, *etc.*

Measuring expression includes determining or detecting the amount of the polypeptide present in a cell or shed by it, as well as measuring the underlying mRNA, where the quantity of mRNA present is considered to reflect the quantity of polypeptide manufactured by the cell. Furthermore, the gene for the NPR-A can be analyzed to determine whether there is a gene defect responsible for aberrant expression or polypeptide activity.

Polypeptide detection can be carried out by any available method, *e.g.*, by Western blots, ELISA, dot blot, immunoprecipitation, RIA, immunohistochemistry, *etc.* For instance, a tissue section can be prepared and labeled with a specific antibody (indirect or direct and visualized with a microscope. Amount of a polypeptide can be quantitated without visualization, *e.g.*, by preparing a lysate of a sample of interest, and then determining by ELISA or Western the amount of polypeptide per quantity of tissue. Antibodies and other specific binding agents can be used. There is no limitation on how detection of NPR-A activity is performed.

Assays can be utilized which permit quantification and/or presence/absence detection of a target nucleic acid (*e.g.*, NPR-A) in a sample. Assays can be performed at the single-cell level, or in a sample comprising many cells, where the assay is "averaging" expression over the entire collection of cells and tissue present in the sample. Any suitable assay format can be used, including, but not limited to, *e.g.*, Southern blot analysis, Northern blot analysis, polymerase chain reaction ("PCR") (*e.g.*, Saiki *et al.*, *Science* 1988, 241, 53; U.S. Pat. Nos. 4,683,195, 4,683,202, and 6,040,166; PCR

Protocols: A Guide to Methods and Applications, Innis *et al.*, eds., Academic Press, New York, 1990), reverse transcriptase polymerase chain reaction ("RT-PCR"), anchored PCR, rapid amplification of cDNA ends ("RACE") (*e.g.*, Schaefer in Gene Cloning and Analysis: Current Innovations, Pages 99-115, 1997), ligase chain reaction ("LCR") (EP 320 308), one-sided PCR (Ohara *et al.*, *Proc. Natl. Acad. Sci.* 1989, 86, 5673-5677), indexing methods (*e.g.*, U.S. Pat. No. 5,508,169), in situ hybridization, differential display (*e.g.*, Liang *et al.*, *Nucl. Acid. Res.* 1993, 21, 3269 3275; U.S. Pat. Nos. 5,262,311, 5,599,672 and 5,965,409; WO97/18454; Prashar and Weissman, *Proc. Natl. Acad. Sci.*, 93:659-663, and U.S. Pat. Nos. 6,010,850 and 5,712,126; Welsh *et al.*, *Nucleic Acid Res.*, 20:4965-4970, 1992, and U.S. Pat. No. 5,487,985) and other RNA fingerprinting techniques, nucleic acid sequence based amplification ("NASBA") and other transcription based amplification systems (*e.g.*, U.S. Pat. Nos. 5,409,818 and 5,554,527; WO 88/10315), polynucleotide arrays (*e.g.*, U.S. Pat. Nos. 5,143,854, 5,424,186; 5,700,637, 5,874,219, and 6,054,270; PCT WO 92/10092; PCT WO 90/15070), Qbeta Replicase (PCT/US87/00880), Strand Displacement Amplification ("SDA"), Repair Chain Reaction ("RCR"), nuclease protection assays, subtraction-based methods, Rapid-Scan, *etc.* Additional useful methods include, but are not limited to, *e.g.*, template-based amplification methods, competitive PCR (*e.g.*, U.S. Pat. No. 5,747,251), redox-based assays (*e.g.*, U.S. Pat. No. 5,871,918), Taqman-based assays (*e.g.*, Holland *et al.*, *Proc. Natl. Acad. Sci.* 1991, 88, 7276-7280; U.S. Pat. Nos. 5,210,015 and 5,994,063), real-time fluorescence-based monitoring (*e.g.*, U.S. Pat. No. 5,928,907), molecular energy transfer labels (*e.g.*, U.S. Pat. Nos. 5,348,853, 5,532,129, 5,565,322, 6,030,787, and 6,117,635; Tyagi and Kramer, *Nature Biotech.*, 14:303-309, 1996). Any method suitable for single cell analysis of gene or protein expression can be used, including in situ hybridization, immunocytochemistry, MACS, FACS, flow cytometry, *etc.* For single cell assays, expression products can be measured using antibodies, PCR, or other types of nucleic acid amplification (*e.g.*, Brady *et al.*, *Methods Mol. & Cell. Biol.* 1990, 2, 17-25; Eberwine *et al.*, *Proc. Natl. Acad. Sci.* 1992, 89, 3010-3014; U.S. Pat. No. 5,723,290). These and other methods can be carried out conventionally, *e.g.*, as described in the mentioned publications.

The terms "transfection", "transformation", and "introduction", and grammatical variations thereof, are used interchangeably herein to refer to the insertion of an

exogenous polynucleotide (*e.g.*, a nucleic acid sequence encoding an NP, or fragment, homolog, or variant thereof, or a nucleic acid sequence encoding an NPR-A, or fragment, homolog, or variant thereof, into a host cell, irrespective of the method used for the insertion, the molecular form of the polynucleotide that is inserted, or the nature of the cell (*e.g.*, prokaryotic or eukaryotic). The insertion of a polynucleotide per se and the insertion of a plasmid or vector comprised of the exogenous polynucleotide are included. The exogenous polynucleotide may be directly transcribed and translated by the cell, maintained as a nonintegrated vector, for example, a plasmid, or alternatively, may be stably integrated into the host genome. Thus, host cells of the invention include those that have been transfected with polynucleotides encoding an NP, or fragment, variant, or homolog thereof; and those that have been transfected with polynucleotides encoding an NPR-A, or fragment, variant, or homolog thereof.

The phrases "isolated" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany the material as it is found in its native state.

An "isolated polynucleotide" that encodes a particular polypeptide refers to a polynucleotide that is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include functionally and/or structurally conservative mutations as defined herein.

The terms "cell" and "cells" are used interchangeably herein to refer to a single cell or plurality of cells (*i.e.*, at least one cell). Typically, host cells used in the methods of the invention are isolated. However, tissues, and genetically modified or transgenic animals may also be utilized.

The terms "comprising", "consisting of" and "consisting essentially of" are defined according to their standard meaning. The terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

As used in this specification, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a cell" includes more than one such cell. Reference to "a receptor" includes more than one such receptor. Reference to "a polynucleotide" includes more than one such polynucleotide. Reference to "a polypeptide" or "agent" includes more than one such polypeptide or agent, and the like.

The practice of the present invention can employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, electrophysiology, and pharmacology, that are within the skill of the art. Such techniques are explained fully in the literature (see, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989); DNA Cloning, Vols. I and II (D. N. Glover ed. 1985); Perbal, B., *A Practical Guide to Molecular Cloning* (1984); the series, *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Transcription and Translation* (Hames *et al.* eds. 1984); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller *et al.* eds. (1987) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); *Scopes, Protein Purification: Principles and Practice* (2nd ed., Springer-Verlag); and *PCR: A Practical Approach* (McPherson *et al.* eds. (1991) IRL Press)).

Example 1—pNP 73-102 inhibits NPRA expression

The structures of ANP and ANP like molecules with their ring-structure and receptors associated with it are well characterized. However, the N-terminal peptides do not have this structure. Neither KP nor NP73-102 was shown to bind ANP receptor NPRA (Mohapatra *et al.*, *J Allergy Clin Immunol*, 2004, 114: 520-526). The receptors for NP-73-102 are not known.

The highest expression of the ANP and ANP receptors is found in neonatal thymus. To test whether the peptide NP73-102 inhibits *in vivo* the ANP cascade, pregnant (12 days) mice were injected *i.p.* with pVAX (vector), or pNP73-102. After 1 day, mice were sacrificed and thymi removed from embryo, were homogenized. Cells were centrifuged and erythrocytes lysed by treating the suspension with ACK buffer. Cells were incubated with anti-NPRA or anti-NPRC antibodies for 1 hour, washed and incubated with PE-conjugated 20 Ab. Levels of NPR's were determined by flow cytometry. The results are shown in Figure 1. The results demonstrate that pNP73-102 inhibited expression of NPRA in thymocytes. Although the mechanism is not clear, this may be due to feedback inhibition at the level intracellular signaling occurring via NPRA.

Example 2—NPRA deficiency decreases pulmonary inflammation

Development and chronicity of cancers has been attributed to the chronic inflammation in the affected organs. ANP was reported to have anti-inflammatory

activity, although signaling through NPR-A is known to cause a number of different biological activity including cell proliferation, immune activation, inflammation and apoptosis. To determine the role of NPR-A signaling in the lung inflammation, groups (n=3) of wild type DBA/2 (wt) and NPR-C (ko) deficient mice and wild type C57/BL6 (wt) and NPR-A (ko) were sensitized with ovalbumin (20 mg/mouse) and after 2 weeks challenged i.n. with ovalbumin (20 mg/mouse). One day later, mice were sacrificed and lung sections were stained with H & E to examine inflammation. As shown in Figures 2A-2D, there was no significant difference in pulmonary inflammation between the wild-type and NPRC deficient mice. In sharp contrast, a comparison between wild-type C57BL6 and NPR-A deficient mice showed that NPR-A deficient mice showed substantially reduced inflammation compared to wild type. These results indicate that ANP-NPR-A signaling is involved in increasing inflammation in the lung.

Example 3—A549 cells transfected with pNP73-102 show a significantly higher level of apoptosis compared control and pANP or pVAX

To determine the effect of overexpression of NP73-102 on proliferation of A549 lung epithelial cells, cells were transfected with either pNP73-102 or vector, pVAX. Cell cycle analysis was performed using propidium iodide (PI) staining and flow cytometry 48 h after transfection. No significant difference was observed between control and pNP73-102-transfected cells in S1, G0-G1 and G2-M stages of cell cycle (data not shown). However, an analysis of apoptosis using flow-cytometry with PI and annexin V, showed that cells transfected with pNP73-102 exhibited significantly higher apoptosis compared to cells transfected with either the control plasmid or a plasmid encoding ANP (Figures 3A-3C). This result was confirmed by (i) staining by TUNEL of A549 cells cultured in 8-chamber slide following a 48-hour transfection with either pANP or pNP73-102 (not shown), (ii) by analysis of PARP cleavage in these cells 48 hours after transfection, which was significantly more prominent in pNP73-102 transfected cells (Figure 3D). The results show that pNP73-102 shows a higher accumulation of apoptotic cells compared to cells transfected with pANP and pVAX controls. Thus, pNP73-102 induces apoptosis of lung adenocarcinoma cells.

In an effort to identify and characterize molecules participating in early signaling pathways, differential gene expression was analyzed using a microarray (AFFYMETRIX). Altered expression of a large number of genes was found, including

genes related to cell growth, cell cycle, and apoptosis. These genes included, among others more than, 6-to 8-fold up-regulation of genes such as Caspase (Casp)-8 and FADD like apoptosis regulator, cyclin E binding protein, CDK inhibitor 1A, CDK7, casp4, casp-10, casp-1, apoptosis facilitator BCL2-like 13 and annexin 43 (data not shown).
5 Together, these studies indicate that pNP73-102 is an inducer of apoptosis in A549 lung adenocarcinoma cells.

Example 4—pNP73-102 decreases tumorigenesis in a colony formation assay by A549

To test the anti-cancer activity of the pNP73-102 construct, a colony forming
10 assay was undertaken. Thus, six cm tissue culture plates were covered with 4 ml of 0.5% soft agar. A549 cells were transfected with pANP, pNP₇₃₋₁₀₂ and pVAX plasmid DNA. After 40 hours of transfection, equal number of cells were suspended in 2 ml of 0.3% soft agar and added to each plate. Cells were plated in duplicate at a density of 2×10^4 cells/dish and incubated for two weeks. Plates were observed and photographed under a
15 microscope. Cell colonies were counted and plotted. The results of one representative experiment of two experiments performed is shown in Figures 5A-5D. The results show that plasmid vector alone caused some reduction in colony formation compared to untransfected control. However, both ANP and pNP₇₃₋₁₀₂ showed substantial reductions in the number of colonies produced compared to vehicle control.

20

Example 5—Chitosan nanoparticle containing pNP₇₃₋₁₀₂ substantially decrease tumor development in the lung

To test the effect of *de novo* expression of pNP₇₃₋₁₀₂, the plasmid was coacervated with chitosan nanoparticles, referred to as CPNP73-102. To examine expression of
25 NP73-102 from CPNP73-102, a construct was developed that carried a C-terminal fusion of marker peptide of FLAG. BALB/c mice were given intranasally the NP73-102-FLAG and the expression of NP73-102-FLAG in the BAL cells after i.n. administration of CPNP73-102-FLAG peptide. A bronchial lavage was performed after 24 hours and lavage cells were stained with either the second antibody control or anti-FLAG antibody
30 (Sigma) and then with DAPI. The results show that intranasal administration induces significant expression of the peptide in the lung cells.

To test whether CPNP73-102 is capable of decreasing tumor formation in the lung, BALB/c nude mice were injected i.v. with 5×10^6 A.549 cells, then treated one day

afterwards and at weekly intervals with CPNP73-102 or control plasmid. After 4 weeks, mice were examined for lung histology. The control animals showed tumors, whereas no tumors were observed in the CpNP73-102-treated group. Sections were also stained with antibodies to cyclinB and to phospho-Bad. The results show that mice treated with CPNP73-102 had no tumors in the lung and did not show any staining for pro-mitotic Cyclin-B and anti-apoptotic marker phospho-Bad. These results indicate that CPNP73-102 has the potential to decrease tumor formation in the lung.

Example 6—Treatment with CPNP73-102 decreases the tumor burden in a spontaneous tumorigenesis model of immunocompetent BALB/c mice

The nude mouse model is deemed to be of less predictive value in terms of translating to human cancer, as mice used are immunodeficient. Therefore, to confirm the results obtained on the potential role of pNP73-102, a syngeneic immunocompetent mouse model of human lung carcinoma was used. For this purpose, Line-1 cell line derived from a bronchioalveolar cell carcinoma (a subtype of lung adenocarcinoma that spontaneously arose in BALB/C mouse (Yuhas *et al.*, *Cancer Research*, 1975, 35:242-244). The cell line forms subcutaneous tumors within 2 to 3 weeks of injection and spontaneously metastasizes to the lung.

To examine whether de novo synthesis of NP73-102 affects tumor development, two groups of BALB/c mice (n=4) were administered with the Line-1 tumor cells (100,000 cells/mouse) at the flanks. One group was administered intranasally with CPNP73-102 the same day, whereas another group was administered with vehicle alone (nanoparticle carrying a plasmid without NP73-102), and the third group was given the saline. Treatment was continued with NP73-102 or controls at weekly intervals for 5 weeks. The tumors were dissected out from each group of mice and photographed (Figures 6A-6C) and the tumor burden was calculated by weighing them on a balance (Figure 6D). The results show that mice administered with CPNP73-102 had significantly decreased tumor burden ($P<0.05$).

Example 7—pNP73-102 induces apoptosis in chemoresistant ovarian cancer cells

The adenocarcinomas of various tissues such as lung, ovary, and breasts have many characteristics that are similar. Chemoresistance is a major therapeutic problem in many of the cancers and the current knowledge on cellular mechanisms involved is

incomplete. Since A549 cells showed differential sensitivity to apoptosis with pVAX and pNP₇₃₋₁₀₂, the effects of pNP₇₃₋₁₀₂ was tested using chemosensitive (OV2008) and chemoresistant (C13) ovarian cancer cells. C-13 and OV2008 ovarian cancer cells were transfected with pNP₇₃₋₁₀₂ or with pVAX as control. Forty-eight hours later, cells were processed to examine apoptosis by TUNEL assay (Figure 7). The results showed that either of the cells when transfected with pVAX did not exhibit any apoptosis. In contrast, both cell lines exhibited apoptosis as evident from TUNEL positive cells. These results indicate that pNP₇₃₋₁₀₂ may induce apoptosis of epithelial adenocarcinomas irrespective of their degree of chemo-sensitivity.

Example 8—MCF-7 breast cancer cells are also affected by NP₇₃₋₁₀₂

The effects of *de novo* synthesis of NP₇₃₋₁₀₂ was examined on the proliferation of the MCF-7 breast cancer cells. Cells were transfected with pVAX, pANP, or pANP₇₃₋₁₀₂. The cells were counted 24 and 48 hours after transfection and their viability was examined by trypan blue staining. The results shown in Figure 8 indicate that there was a substantial reduction of viable cell numbers in cells transfected with pNP₇₃₋₁₀₂ compared to cells transfected with pANP or control empty vector. To further verify whether this is due to a defect in cell cycle or induction of apoptosis, a cell cycle analysis was undertaken. MCF-7 cells were transfected with pVAX or pANP₇₃₋₁₀₂ and DNA analysis was undertaken by PI staining 48 hours after transfection. Cells transfected with empty vector plasmid as control showed 37.99% cells in G0-G1, 11.28% in G2-M and 50.73% cells in G2-G1 phase. In contrast, cells transfected with pANP₇₃₋₁₀₂ showed 66.01% cells in G0-G1, 7.07% in G2-M, and 26.91% cells in G2-G1 phase. Transfection with pANP showed results similar to the pNP₇₃₋₁₀₂. These results indicate that both pANP and pNP₇₃₋₁₀₂ expression arrests cells in G0-G1 and blocks progression to S phase, suggesting that treatment with pANP and pNP₇₃₋₁₀₂ or the corresponding peptides may be useful in breast cancer patients.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures, tables, and sequences, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

What is claimed is:

1. A method of treating or reducing the onset of an inflammatory or cell proliferation disorder, comprising administering a natriuretic hormone peptide (NP), or a polynucleotide encoding NP and an operably-linked promoter sequence, to a patient in need thereof.

2. The method of claim 1, wherein said administering comprises administering the NP to the patient, and wherein the NP comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:6, or a biologically active fragment or homolog of any of the foregoing.

3. The method of claim 1, wherein said administering comprises administering the polynucleotide encoding NP to the patient, and wherein the NP comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, or a biologically active fragment or homolog of any of the foregoing.

4. The method of claim 1, wherein said administering is by a route selected from the group consisting of oral, intramuscular, parenteral, intravenous, and intranasal.

5. The method of claim 1, wherein the NP or the polynucleotide is administered with a pharmaceutically acceptable carrier.

6. The method of claim 1, wherein said administering comprises administering the polynucleotide to the patient, and wherein the polynucleotide is contained within an expression vector.

7. The method of claim 6, wherein the expression vector is a DNA plasmid or virus.

8. The method of claim 1, wherein said administering comprises administering the polynucleotide to the patient, and wherein the nucleic acid sequence is administered with chitosan.

9. The method of claim 1, wherein the inflammatory or proliferation disorder is cancer.

10. The method of claim 1, wherein the patient is suffering from one or more tumors, and wherein the NP causes regression of one or more of the tumors in the patient.

11. The method of claim 1, wherein the NP reduces tumor growth and metastasis in the patient.

12. The method of claim 1, wherein the patient is suffering from the inflammatory or cell proliferation disorder.

13. The method of claim 1, wherein the patient is human.

14. A method for reducing the activity of atrial natriuretic peptide receptor-A (NPR-A) in cells *in vitro* or *in vivo*, comprising administering an effective amount of an agent that reduces NPR-A activity to the cells.

15. The method of claim 14, wherein the agent is selected from the group consisting of a polypeptide, polynucleotide, and small molecule.

16. The method of claim 14, wherein the agent comprises a natriuretic hormone peptide (NP), or a polynucleotide encoding NP and an operably-linked promoter sequence.

17. A pharmaceutical composition comprising an agent that reduces the activity of atrial natriuretic peptide receptor-A (NPR-A), and an anti-cancer agent.

18. The pharmaceutical composition of claim 17, wherein said anti-cancer agent comprises at least one agent selected from the group consisting of a chemotherapeutic agent,

a matrix metalloproteinase (MMP)-inhibitor, an angiogenesis inhibitor, a Bcl-2 antisense oligonucleotide, a PSA based vaccine, a PDGF receptor inhibitor, a microtubule stabilizer, and a pro-apoptotic agent.

19. The pharmaceutical composition of claim 17, wherein the agent comprises a natriuretic hormone peptide (NP), or a polynucleotide encoding NP and an operably-linked promoter sequence.

20. A method for identifying an agent useful for treating an inflammatory or cell proliferation disorder, comprising determining whether the agent reduces the activity of atrial natriuretic peptide receptor-A (NPR-A).

21. The method of claim 20, wherein said determining comprises providing a host cell that produces NPR-A; contacting the host cell with the candidate agent *in vitro* or *in vivo*; and determining whether the candidate agent reduces intracellular cGMP.

1/11

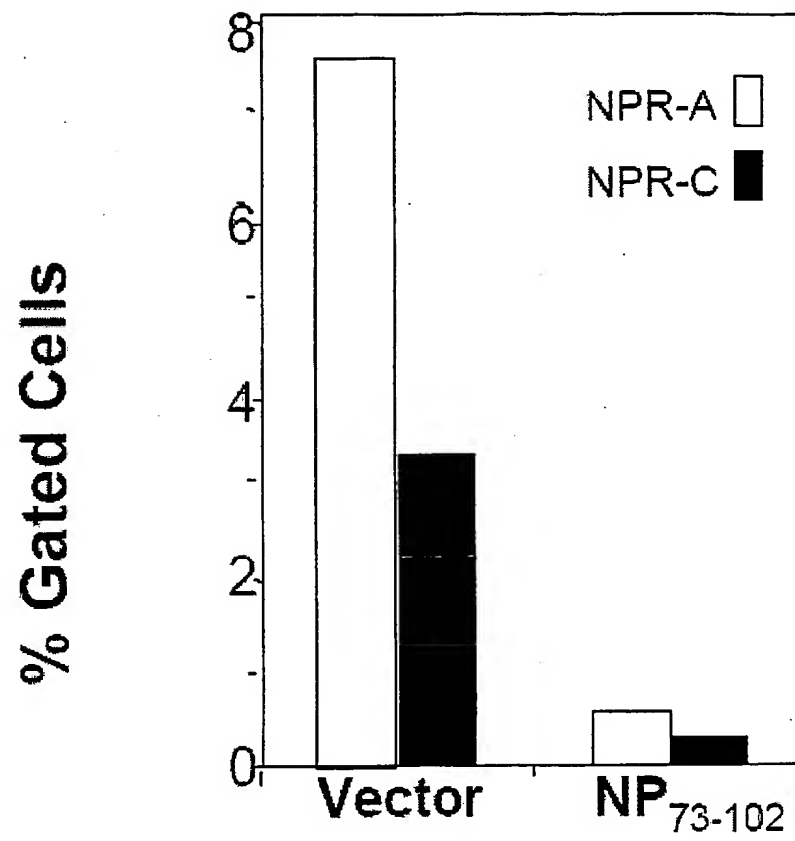


FIG. 1

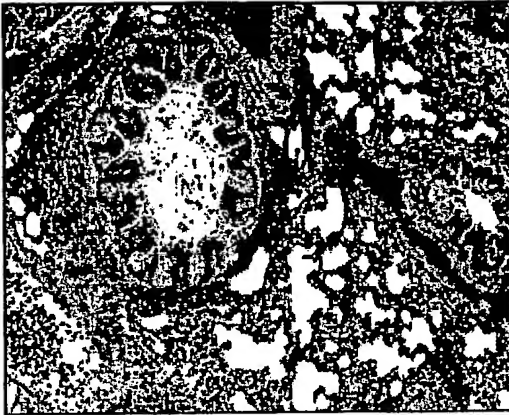


FIG. 2A



FIG. 2B



FIG. 2C



FIG. 2D

3/11

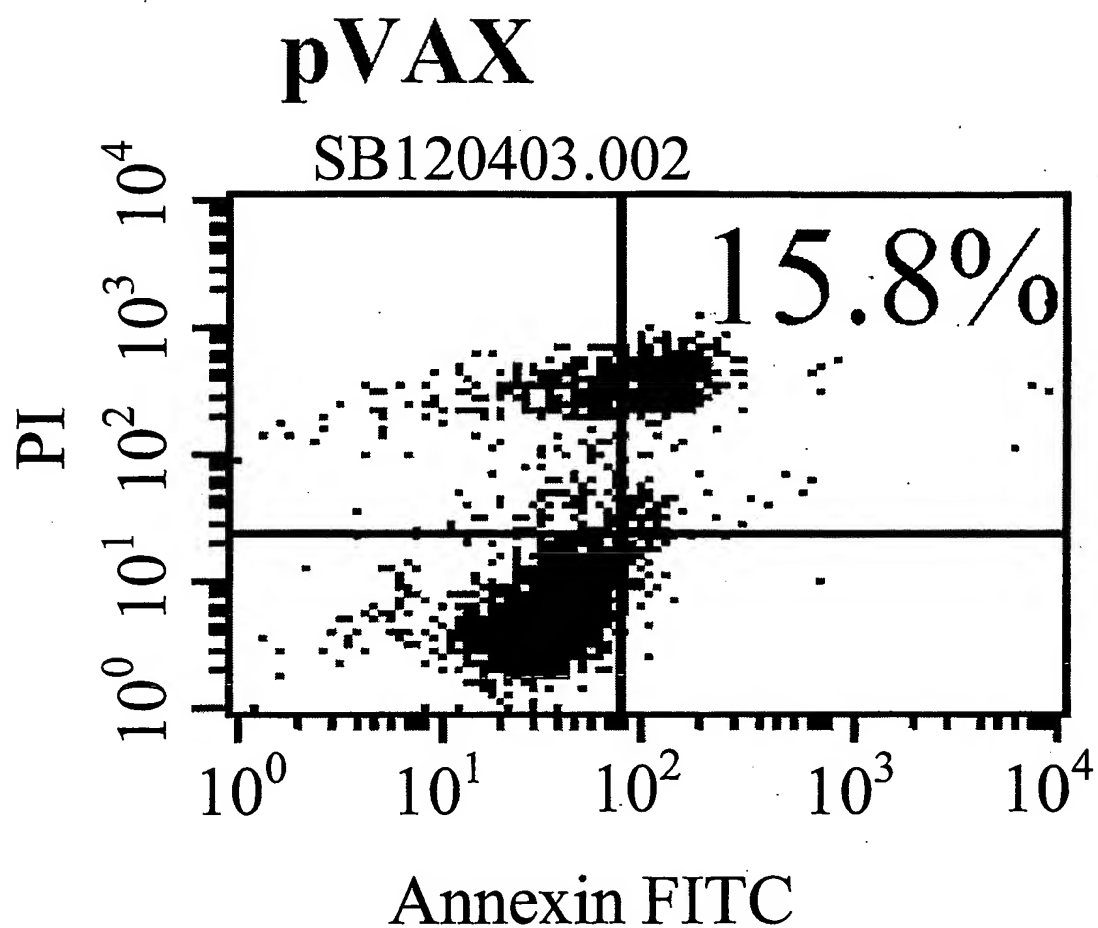


FIG. 3A

4/11

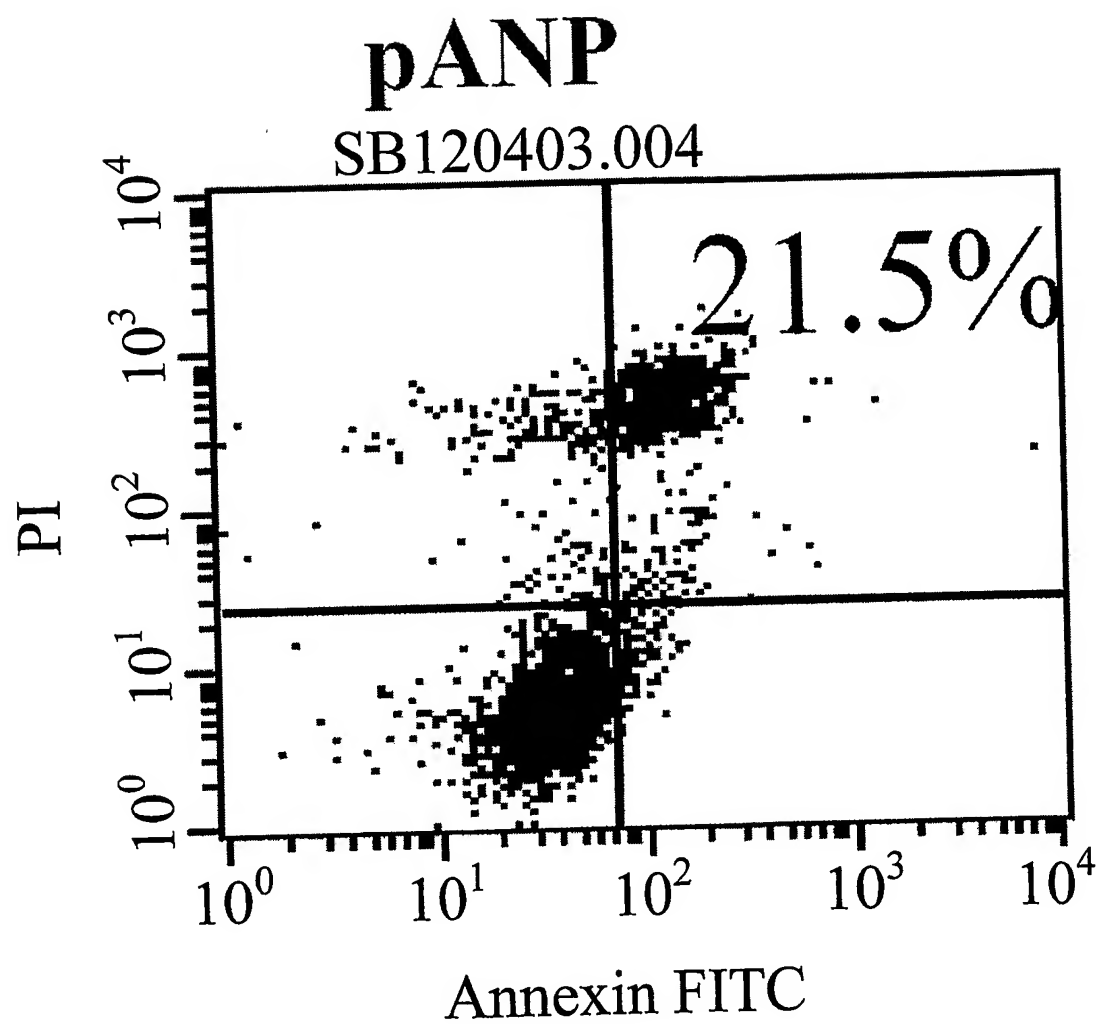


FIG. 3B

5/11

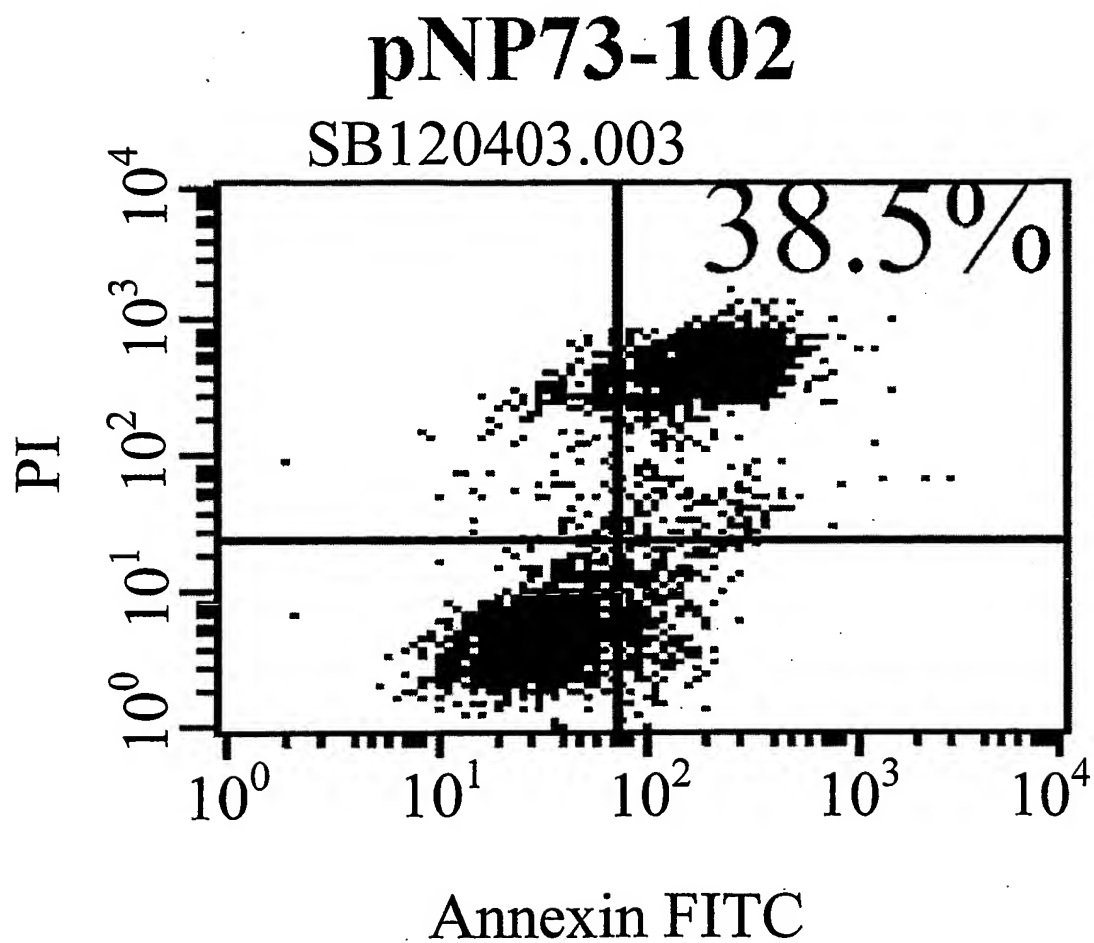


FIG. 3C

6/11

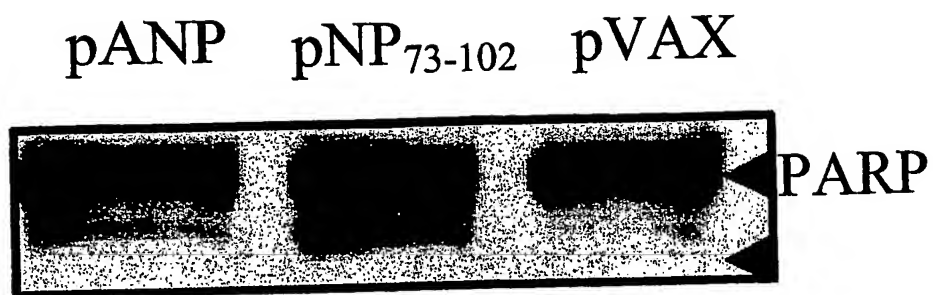


FIG. 3D

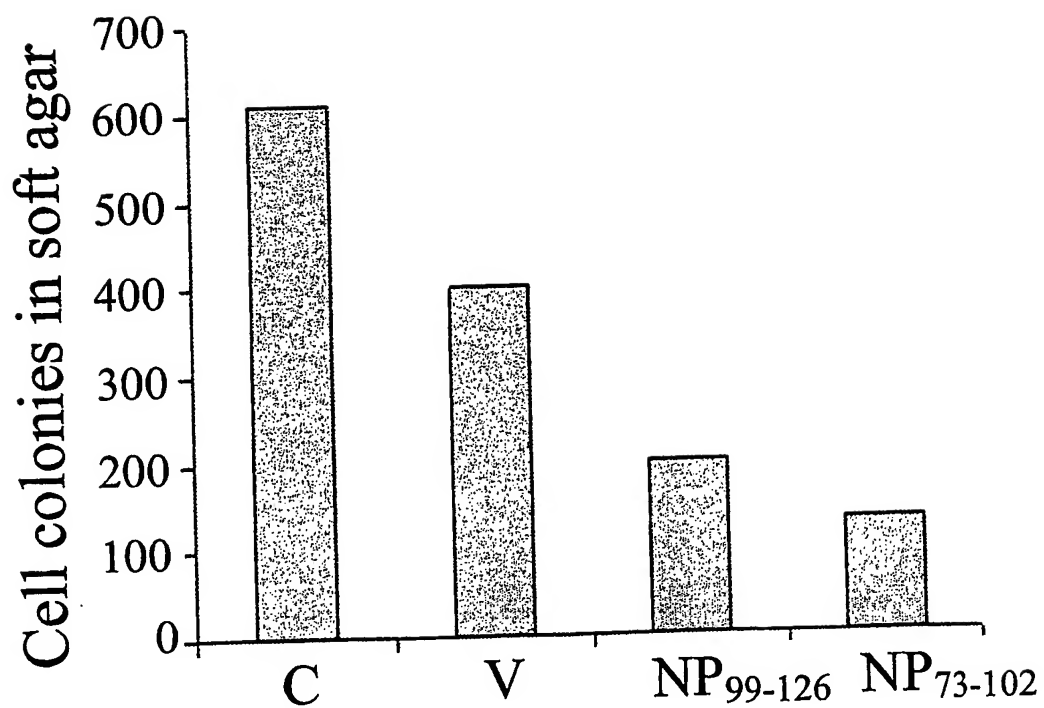


FIG. 4

7/11

FIG. 5A

DAPI

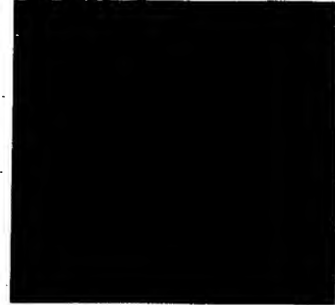


FIG. 5B

Control

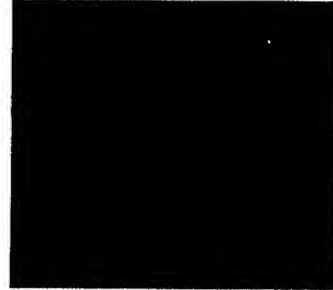
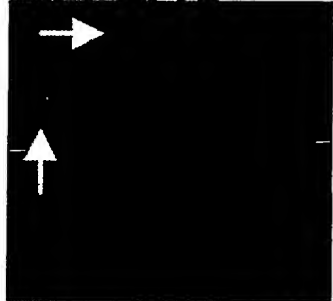
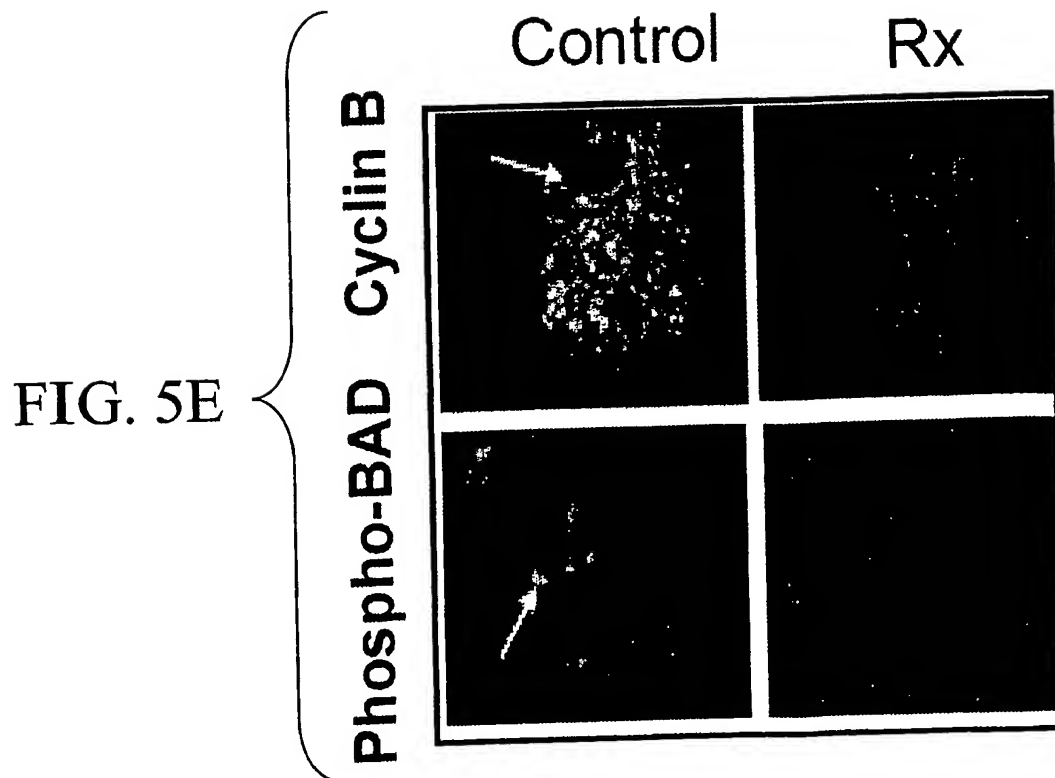
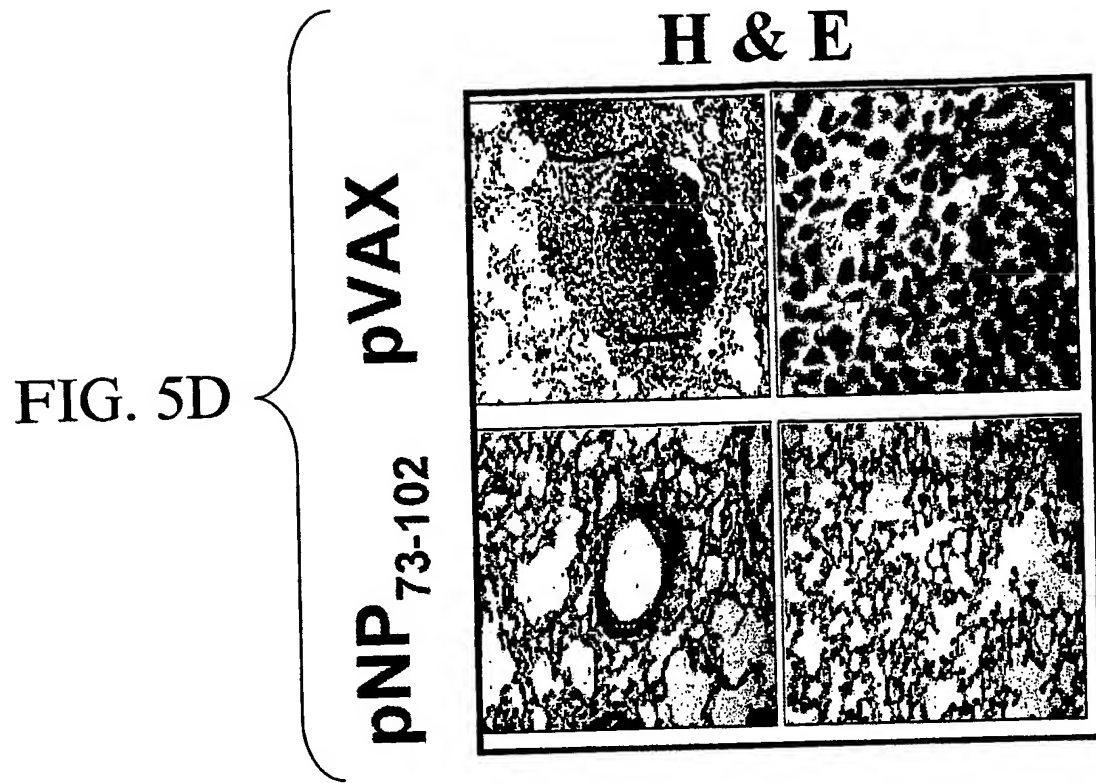


FIG. 5C

 α -FLAG



9/11

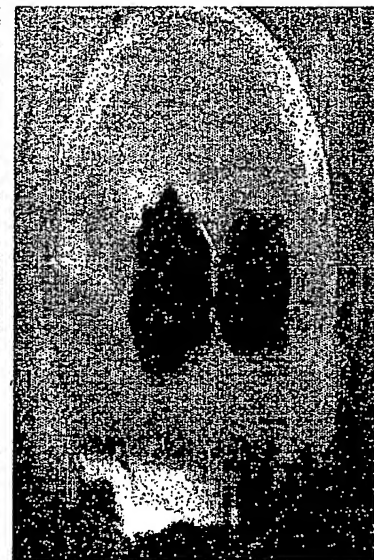
FIG. 6A

**Control**

FIG. 6B

**Vehicle**

FIG. 6C

**NP₇₃₋₁₀₂**

10/11

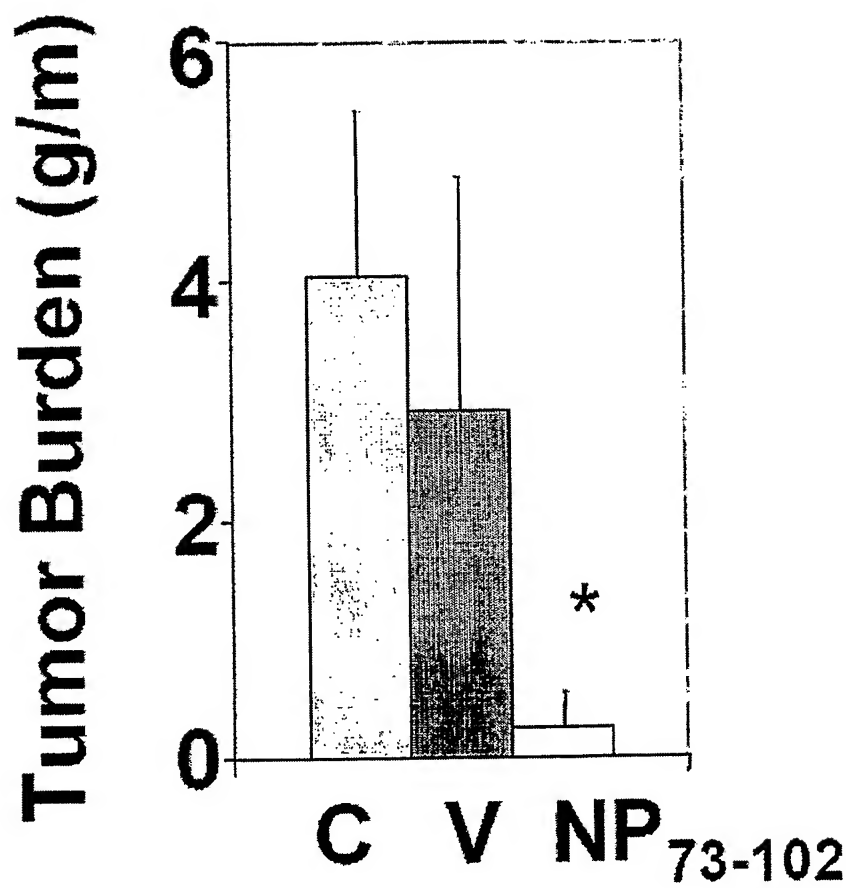


FIG. 6D

11/11

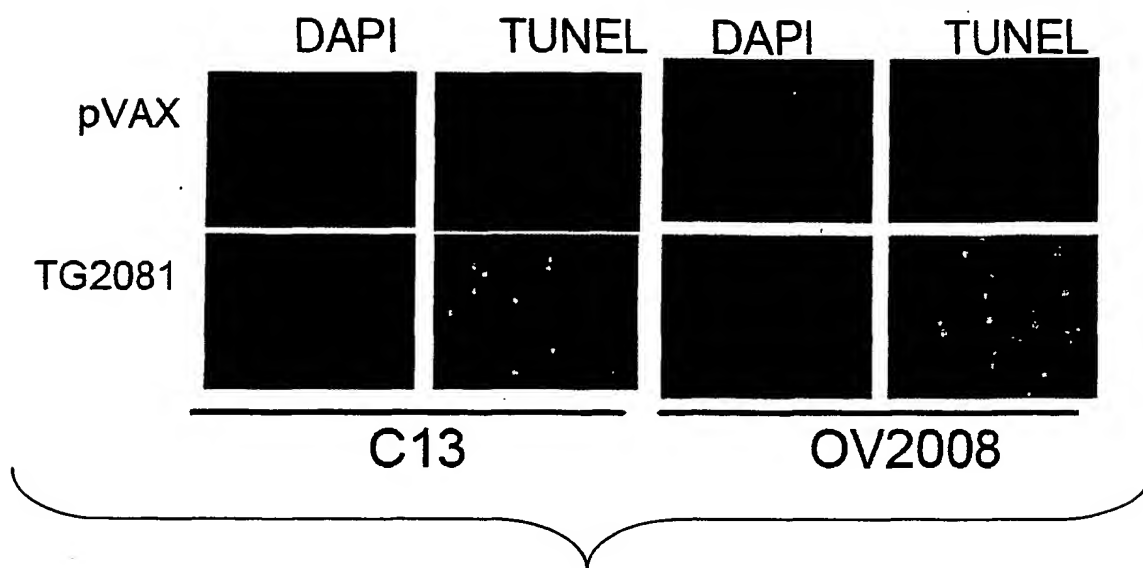


FIG. 7

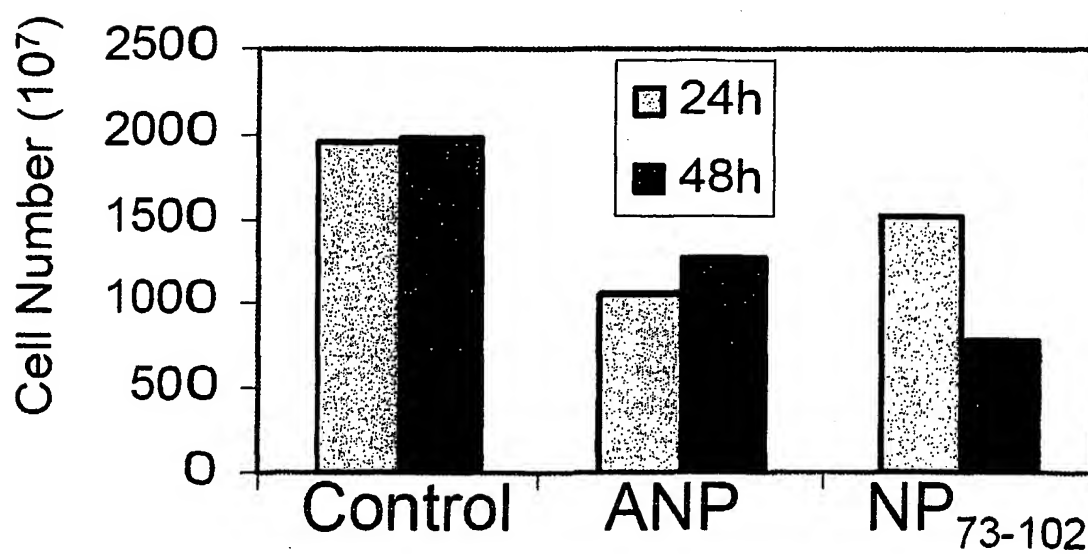


FIG. 8

SEQUENCE LISTING

<110> University of South Florida
Mohapatra, Shyam

<120> Materials and Methods for Treatment of Inflammatory and Cell
Proliferation Disorders

<130> USF-215PTCZ

<150> 60/521,072

<151> 2004-02-17

<160> 22

<170> PatentIn version 3.2

<210> 1

<211> 30

<212> PRT

<213> Homo sapiens

<400> 1

Asn	Pro	Met	Tyr	Asn	Ala	Val	Ser	Asn	Ala	Asp	Leu	Met	Asp	Phe	Lys
1				5				10						15	

Asn	Leu	Leu	Asp	His	Leu	Glu	Glu	Lys	Met	Pro	Leu	Glu	Asp
			20					25					30

<210> 2

<211> 37

<212> PRT

<213> Homo sapiens

<400> 2

Glu	Val	Val	Pro	Pro	Gln	Val	Leu	Ser	Glu	Pro	Asn	Glu	Glu	Ala	Gly
1				5				10						15	

Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	Glu	Val
			20					25					30		

Ser	Pro	Ala	Gln	Arg
			35	

<210> 3

<211> 20

<212> PRT

<213> Homo sapiens

<400> 3

Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser	Lys	Leu	Arg	Ala	Leu	Leu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

2

1 5 10 15

Thr Ala Pro Arg
20

<210> 4
<211> 28
<212> PRT
<213> Homo sapiens

<400> 4

Ser Leu Arg Arg Ser Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly
1 5 10 15

Ala Gln Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr
20 25

<210> 5
<211> 29
<212> PRT
<213> Mus musculus

<400> 5

Gly Ser Pro Trp Asp Pro Ser Asp Arg Ser Ala Leu Leu Lys Ser Lys
1 5 10 15

Leu Arg Ala Leu Leu Ala Gly Pro Arg Ser Leu Arg Arg
20 25

<210> 6
<211> 37
<212> PRT
<213> Mus musculus

<400> 6

Val Ser Asn Thr Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu
1 5 10 15

Glu Glu Lys Met Pro Val Glu Asp Glu Val Met Pro Pro Gln Ala Leu
20 25 30

Ser Glu Gln Thr Glu
35

<210> 7
<211> 151
<212> PRT

3

<213> Homo sapiens

<400> 7

Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
 1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
 20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
 35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
 50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
 65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
 85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
 100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
 115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
 130 135 140

Gly Cys Asn Ser Phe Arg Tyr
 145 150

<210> 8

<211> 35

<212> DNA

<213> Mus musculus

<400> 8

gacggcaagc ttactatggg cagcccctgg gaccc

35

<210> 9

<211> 33

<212> DNA

<213> Mus musculus

<400> 9

acccccctcg agttattatc ttogtaggct ccg 33

<210> 10
<211> 33
<212> DNA
<213> Mus musculus

<400> 10
aatcctaagc ttagtatggt gtccaacaca gat 33

<210> 11
<211> 41
<212> DNA
<213> Mus musculus

<400> 11
tgcgaactcg agttactcag tctgctcact cagggcctgc g 41

<210> 12
<211> 93
<212> DNA
<213> Mus musculus

<400> 12
atgggcagcc cctgggaccc ctccgataga tctgccctct tgaaaagcaa actgagggct 60
ctgctcgctg gccctcggag cctacgaaga taa 93

<210> 13
<211> 117
<212> DNA
<213> Mus musculus

<400> 13
atggtgtcca acacagatct gatggatttc aagaacctgc tagaccacct ggaggagaag 60
atgccggtag aagatgaggt catgcccccg caggccctga gtgagcagac tgagtaa 117

<210> 14
<211> 845
<212> DNA
<213> Homo sapiens

<400> 14
tggcgaggga cagacgtagg ccaagagagg ggaaccagag aggaaccaga ggggagagac 60
agagcagcaa gcagtggatt gtccttgac gacgccagca tgagctcctt ctccaccacc 120
accgtgagct tcctcctttt actggcattc cagctcctag gtcagaccag agctaataccc 180
atgtacaatg ccgtgtccaa cgcagacctg atggatttca agaatttgct ggaccatttg 240
gaagaaaaga tgcctttaga agatgaggtc gtgccccac aagtgctcag tgagccgaat 300

gaagaagcgg gggctgctct cagccccctc cctgaggtgc ctccctggac cggggaagtc 360
 agcccagccc agagagatgg aggtgccctc gggcggggcc cctgggactc ctctgatcga 420
 tctgccctcc taaaaagcaa gctgagggcg ctgctcactg cccctcggag cctgcggaga 480
 tccagctgct tcgggggcag gatggacagg attggagccc agagcggact gggctgtaac 540
 agcttccggt actgaagata acagccaggg aggacaagca gggctgggcc tagggacaga 600
 ctgcaagagg ctctgtccc ctgggggtctc tgctgcattt gtgtcatctt gttgccatgg 660
 agttgtgatc atcccatcta agctgcagct tcctgtcaac acttctcaca tcttatgcta 720
 actgtagata aagtggtttg atggtgactt cctcgcctct cccaccccat gcattaaatt 780
 ttaaggtaga acctcacctg ttactgaaag tggtttgaaa gtgaataaac ttcagcacca 840
 tggac 845

<210> 15
 <211> 2583
 <212> DNA
 <213> Homo sapiens

<400> 15
 ggatccattt gtctcgggct gctggctgcc tgccatttcc tcctctccac ccttatttgg 60
 aggccctgac agctgagcca caaacaacc aggggagctg ggcaccagca agcgtcacc 120
 tctgtttccc cgacgggtac cagcgtcag gagaaagaat cctgaggcac ggcggtgaga 180
 taaccaagga ctctttttta ctcttctcac accttgaag tgggagcctc ttgagtcaaa 240
 tcagtaagaa tgcggctctt gcagctgagg gtctgggggg ctggtggggc tgccaaggc 300
 agagaggggc tgtgacaagc cctgcggatg ataactttaa aagggcatct cctgctggct 360
 tctcacttgg cagctttatc actgcaagtg acagaatggg gagggttctg tctctcctgc 420
 gtgcttgagg agctgggggg ctataaaaag aggcggcact gggcagctgg gagacagga 480
 cagacgtagg ccaagagagg ggaaccagag aggaaccaga ggggagagac agagcagcaa 540
 gcagtggatt gtccttgac gacgccagca tgagctcctt ctccaccacc accgtgagct 600
 tcctcctttt actggcattc cagctcctag gtcagaccag agctaattcc atgtacaatg 660
 ccgtgtccaa cgcagacctg atggatttca aggtagggcc aggaaagcgg gtgcagtctg 720
 gggccagggg gctttctgat gctgtgctca ctctcttga tttcctcaa gtcagtggg 780
 tttatccctt tcctgtatt ttcttttct aaagaatttg ctggaccatt tggaagaaaa 840
 gatgccttta gaagatgagg tcgtgcccc acaagtgtc agtgagccga atgaagaagc 900
 gggggctgct ctacagcccc tcctgagggt gcctccctgg accggggaag tcagcccagc 960

ccagagagat ggaggtgccc tcgggcgggg cccctgggac tcctctgac gatctgccct 1020
cctaaaaagc aagctgaggg cgctgctcac tgcccctcg agcctgcgga gatccagctg 1080
cttcgggggc aggatggaca ggattggagc ccagagcgga ctgggctgta acagcttccg 1140
ggtaagagga actggggatg gaaatgggat gggatggaca ctactgggag acaccttcag 1200
caggaaaggg accaatgcag aagctcattc cctctcaagt ttctgcccc aaccccagag 1260
tgccccatgg gtgtcaggac atgccatcta ttgtccttag ctagtctgct gagaaaatgc 1320
ttaaaaaaaaa aagggggggg gctgggcacg gtcgtcacgc ctgtaatccc agcactttgg 1380
gaggccaggc agcggatcat gaggtcaaga gatcaagact atcctggcca acatggtgaa 1440
accccagctc tactaaaaat acaaaaatta gctgggtgtg tggcgggcac ctgtactctc 1500
agctacttgg gaggctgagg caggagaatc acttgaaccc aggaggcaga ggttgcagtg 1560
agcagagatc acgccactgc agtccagcct aggtgataga gcgagactgt ctcaaaaaaa 1620
aaaaaaaaag gccaggcgcg gtggctcacg cctgtaatcc cagcgctttg ggaggccaag 1680
gcgggtggat cacgaggtca ggagatggag accatcctgg ctaacacggg gaaaccccg 1740
ctctactaaa aatacaaaaa attagccagg cgtggtggca ggcgcctgta agtcctagct 1800
actccggagg ctgaggcagg agaattggcg gaacccggga ggcggagctt gcagtgagca 1860
gagatggcac cactgcactc cagcctgggc gacagagcaa gactccgtct caaaaaaaaa 1920
aaaaaaaaaa gcaactgcc atagcactgg gaaattaaaa tattcataga gccaaagttat 1980
ctttgcatgg ctgattagca gttcatattc ctcccagaa ttgcaagatc ctgaagggct 2040
taagtgaat ttactctgat gagtaacttg cttatcaatt catgaagctc agagggtcat 2100
caggctgggg tgggggccgg tgggaagcag gtggtcagta atcaagttca gaggatgggc 2160
acactcatatc atgaagctga cttttccagg acagccaggc caccaagcca gatatgtctg 2220
tggtctcttt gcagtactga agataacagc caggaggagc aagcagggct ggcctaggg 2280
acagactgca agaggctcct gtcccctggg gtctctgctg catttggtgc atcttggtgc 2340
catggagttg tgatcatccc atctaagctg cagcttcctg tcaacacttc tcacatctta 2400
tgctaactgt agataaagtg gtttgatggg gacttcctcg cctctccac cccatgcatt 2460
aaattttaag gtagaacctc acctgttact gaaagtgggt tgaaagtga taaacttcag 2520
caccatggac agaagacaaa tgcctgcgtt ggtgtgcttt ctttcttctt gggaagagaa 2580
ttc 2583

<210> 16

<211> 152

<212> PRT
 <213> Mus musculus

<400> 16

Met Gly Ser Phe Ser Ile Thr Leu Gly Phe Phe Leu Val Leu Ala Phe
 1 5 10 15

Trp Leu Pro Gly His Ile Gly Ala Asn Pro Val Tyr Ser Ala Val Ser
 20 25 30

Asn Thr Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu Glu
 35 40 45

Lys Met Pro Val Glu Asp Glu Val Met Pro Pro Gln Ala Leu Ser Glu
 50 55 60

Gln Thr Glu Glu Ala Gly Ala Ala Leu Ser Ser Leu Pro Glu Val Pro
 65 70 75 80

Pro Trp Thr Gly Glu Val Asn Pro Pro Leu Arg Asp Gly Ser Ala Leu
 85 90 95

Gly Arg Ser Pro Trp Asp Pro Ser Asp Arg Ser Ala Leu Leu Lys Ser
 100 105 110

Lys Leu Arg Ala Leu Leu Ala Gly Pro Arg Ser Leu Arg Arg Ser Ser
 115 120 125

Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Gln Ser Gly Leu Gly
 130 135 140

Cys Asn Ser Phe Arg Tyr Arg Arg
 145 150

<210> 17
 <211> 878
 <212> DNA
 <213> Mus musculus

<220>
 <221> misc_feature
 <222> (81)..(539)
 <223> coding sequence for preproANP

<400> 17
 caaaagctga gagagagaga gaaagaaacc agagtgggca gagacagcaa acatcagatc 60

```

gtgccccgac ccacgccagc atgggctcct tctccatcac cctgggcttc ttcctcgtct 120
tggccttttg gcttccaggc catattggag caaatcctgt gtacagtgcg gtgtccaaca 180
cagatctgat ggatttcaag aacctgctag accacctgga ggagaagatg ccggtagaag 240
atgaggtcat gccccgcag gccctgagtg agcagactga ggaagcaggg gccgcactta 300
gtccctccc cgaggtgcct ccctggactg gggaggtcaa cccacctctg agagacggca 360
gtgctctagg gcgcagcccc tgggaccct ccatagatc tgccctcttg aaaagcaaac 420
tgagggctct gctcgtcggc cctcggagcc tacgaagatc cagctgcttc gggggtagga 480
ttgacaggat tggagcccag agtggactag gctgcaacag cttccggtac cgaagataac 540
agccaaggag gaaaaggcag tcgattctgc ttgagcagat cgcaaaagat cctaagccct 600
tgtggtgtgt cagcgagctt ggtcacattg ccactgtggc gtggtgaaca ccctcctgga 660
gctgcggctt cctgccttca tctatcacga tcgatgttaa atgtagatga gtggtctagt 720
ggggtcttgc ctctcccact ctgcatatta aggtagatcc tcaccctttt cagaaagcag 780
ttggaaaaaa aaaaaaagaa taaacttcag caccaaggac agacgccgag gccctgatgt 840
gcttcttttg cttctgccct cagttctttg ctctcccc 878

```

<210> 18
 <211> 1061
 <212> PRT
 <213> Homo sapiens

<400> 18

Met Pro Gly Pro Arg Arg Pro Ala Gly Ser Arg Leu Arg Leu Leu Leu
 1 5 10 15

Leu Leu Leu Leu Pro Pro Leu Leu Leu Leu Arg Gly Ser His Ala
 20 25 30

Gly Asn Leu Thr Val Ala Val Val Leu Pro Leu Ala Asn Thr Ser Tyr
 35 40 45

Pro Trp Ser Trp Ala Arg Val Gly Pro Ala Val Glu Leu Ala Leu Ala
 50 55 60

Gln Val Lys Ala Arg Pro Asp Leu Leu Pro Gly Trp Thr Val Arg Thr
 65 70 75 80

Val Leu Gly Ser Ser Glu Asn Ala Leu Gly Val Cys Ser Asp Thr Ala
 85 90 95

Ala Pro Leu Ala Ala Val Asp Leu Lys Trp Glu His Asn Pro Ala Val
 100 105 110

Phe Leu Gly Pro Gly Cys Val Tyr Ala Ala Ala Pro Val Gly Arg Phe
 115 120 125

Thr Ala His Trp Arg Val Pro Leu Leu Thr Ala Gly Ala Pro Ala Leu
 130 135 140

Gly Phe Gly Val Lys Asp Glu Tyr Ala Leu Thr Thr Arg Ala Gly Pro
 145 150 155 160

Ser Tyr Ala Lys Leu Gly Asp Phe Val Ala Ala Leu His Arg Arg Leu
 165 170 175

Gly Trp Glu Arg Gln Ala Leu Met Leu Tyr Ala Tyr Arg Pro Gly Asp
 180 185 190

Glu Glu His Cys Phe Phe Leu Val Glu Gly Leu Phe Met Arg Val Arg
 195 200 205

Asp Arg Leu Asn Ile Thr Val Asp His Leu Glu Phe Ala Glu Asp Asp
 210 215 220

Leu Ser His Tyr Thr Arg Leu Leu Arg Thr Met Pro Arg Lys Gly Arg
 225 230 235 240

Val Ile Tyr Ile Cys Ser Ser Pro Asp Ala Phe Arg Thr Leu Met Leu
 245 250 255

Leu Ala Leu Glu Ala Gly Leu Cys Gly Glu Asp Tyr Val Phe Phe His
 260 265 270

Leu Asp Ile Phe Gly Gln Ser Leu Gln Gly Gly Gln Gly Pro Ala Pro
 275 280 285

Arg Arg Pro Trp Glu Arg Gly Asp Gly Gln Asp Val Ser Ala Arg Gln
 290 295 300

Ala Phe Gln Ala Ala Lys Ile Ile Thr Tyr Lys Asp Pro Asp Asn Pro
 305 310 315 320

Glu Tyr Leu Glu Phe Leu Lys Gln Leu Lys His Leu Ala Tyr Glu Gln
 325 330 335

Phe Asn Phe Thr Met Glu Asp Val Leu Val Asn Thr Ile Pro Ala Ser
340 345 350

Phe His Asp Gly Leu Leu Leu Tyr Ile Gln Ala Val Thr Glu Thr Leu
355 360 365

Ala His Gly Gly Thr Val Thr Asp Gly Glu Asn Ile Thr Gln Arg Met
370 375 380

Trp Asn Arg Ser Phe Gln Gly Val Thr Gly Tyr Leu Lys Ile Asp Ser
385 390 395 400

Ser Gly Asp Arg Glu Thr Asp Phe Ser Leu Trp Asp Met Asp Pro Glu
405 410 415

Asn Gly Ala Phe Arg Val Val Leu Asn Tyr Asn Gly Thr Ser Gln Glu
420 425 430

Leu Val Ala Val Ser Gly Arg Lys Leu Asn Trp Pro Leu Gly Tyr Pro
435 440 445

Pro Pro Asp Ile Pro Lys Cys Gly Phe Asp Asn Glu Asp Pro Ala Cys
450 455 460

Asn Gln Asp His Leu Ser Thr Leu Glu Val Leu Ala Leu Val Gly Ser
465 470 475 480

Leu Ser Leu Leu Gly Ile Leu Ile Val Ser Phe Phe Ile Tyr Arg Lys
485 490 495

Met Gln Leu Glu Lys Glu Leu Ala Ser Glu Leu Trp Arg Val Arg Trp
500 505 510

Glu Asp Val Glu Pro Ser Ser Leu Glu Arg His Leu Arg Ser Ala Gly
515 520 525

Ser Arg Leu Thr Leu Ser Gly Arg Gly Ser Asn Tyr Gly Ser Leu Leu
530 535 540

Thr Thr Glu Gly Gln Phe Gln Val Phe Ala Lys Thr Ala Tyr Tyr Lys
545 550 555 560

Gly Asn Leu Val Ala Val Lys Arg Val Asn Arg Lys Arg Ile Glu Leu

565

570

575

Thr Arg Lys Val Leu Phe Glu Leu Lys His Met Arg Asp Val Gln Asn
 580 585 590

Glu His Leu Thr Arg Phe Val Gly Ala Cys Thr Asp Pro Pro Asn Ile
 595 600 605

Cys Ile Leu Thr Glu Tyr Cys Pro Arg Gly Ser Leu Gln Asp Ile Leu
 610 615 620

Glu Asn Glu Ser Ile Thr Leu Asp Trp Met Phe Arg Tyr Ser Leu Thr
 625 630 635 640

Asn Asp Ile Val Lys Gly Met Leu Phe Leu His Asn Gly Ala Ile Cys
 645 650 655

Ser His Gly Asn Leu Lys Ser Ser Asn Cys Val Val Asp Gly Arg Phe
 660 665 670

Val Leu Lys Ile Thr Asp Tyr Gly Leu Glu Ser Phe Arg Asp Leu Asp
 675 680 685

Pro Glu Gln Gly His Thr Val Tyr Ala Lys Lys Leu Trp Thr Ala Pro
 690 695 700

Glu Leu Leu Arg Met Ala Ser Pro Pro Val Arg Gly Ser Gln Ala Gly
 705 710 715 720

Asp Val Tyr Ser Phe Gly Ile Ile Leu Gln Glu Ile Ala Leu Arg Ser
 725 730 735

Gly Val Phe His Val Glu Gly Leu Asp Leu Ser Pro Lys Glu Ile Ile
 740 745 750

Glu Arg Val Thr Arg Gly Glu Gln Pro Pro Phe Arg Pro Ser Leu Ala
 755 760 765

Leu Gln Ser His Leu Glu Glu Leu Gly Leu Leu Met Gln Arg Cys Trp
 770 775 780

Ala Glu Asp Pro Gln Glu Arg Pro Pro Phe Gln Gln Ile Arg Leu Thr
 785 790 795 800

Leu Arg Lys Phe Asn Arg Glu Asn Ser Ser Asn Ile Leu Asp Asn Leu
805 810 815

Leu Ser Arg Met Glu Gln Tyr Ala Asn Asn Leu Glu Glu Leu Val Glu
820 825 830

Glu Arg Thr Gln Ala Tyr Leu Glu Glu Lys Arg Lys Ala Glu Ala Leu
835 840 845

Leu Tyr Gln Ile Leu Pro His Ser Val Ala Glu Gln Leu Lys Arg Gly
850 855 860

Glu Thr Val Gln Ala Glu Ala Phe Asp Ser Val Thr Ile Tyr Phe Ser
865 870 875 880

Asp Ile Val Gly Phe Thr Ala Leu Ser Ala Glu Ser Thr Pro Met Gln
885 890 895

Val Val Thr Leu Leu Asn Asp Leu Tyr Thr Cys Phe Asp Ala Val Ile
900 905 910

Asp Asn Phe Asp Val Tyr Lys Val Glu Thr Ile Gly Asp Ala Tyr Met
915 920 925

Val Val Ser Gly Leu Pro Val Arg Asn Gly Arg Leu His Ala Cys Glu
930 935 940

Val Ala Arg Met Ala Leu Ala Leu Leu Asp Ala Val Arg Ser Phe Arg
945 950 955 960

Ile Arg His Arg Pro Gln Glu Gln Leu Arg Leu Arg Ile Gly Ile His
965 970 975

Thr Gly Pro Val Cys Ala Gly Val Val Gly Leu Lys Met Pro Arg Tyr
980 985 990

Cys Leu Phe Gly Asp Thr Val Asn Thr Ala Ser Arg Met Glu Ser Asn
995 1000 1005

Gly Glu Ala Leu Lys Ile His Leu Ser Ser Glu Thr Lys Ala Val
1010 1015 1020

Leu Glu Glu Phe Gly Gly Phe Glu Leu Glu Leu Arg Gly Asp Val
1025 1030 1035

Glu Met Lys Gly Lys Gly Lys Val Arg Thr Tyr Trp Leu Leu Gly
 1040 1045 1050

Glu Arg Gly Ser Ser Thr Arg Gly
 1055 1060

<210> 19
 <211> 4246
 <212> DNA
 <213> Homo sapiens

<400> 19
 ggttccctcc ggatagccgg agacttgggc cggccggacg ccccttctgg cacactccct 60
 ggggcaggcg ctacgcacg ctacaaacac acactcctct ttctccctc gcgcgcctc 120
 tctcatcctt cttcacgaag cgctcactcg caccctttct ctctctctct ctctctctaa 180
 cacgcacgca cactcccagt tggtcacact cgggtcctct ccagcccagc gttctcctgg 240
 caccacactg ctccgcggcg ccctgcgcgc cccctcgggt cgcgcccctt gcgctctcgg 300
 cccagaccgt cgcagctaca gggggcctcg agccccgggg tgagcgtccc cgtcccgtc 360
 ctgctccttc ccatagggac gcgcctgatg cctgggaccg gccgctgagc ccaaggggac 420
 cgaggaggcc atggtaggag cgctcgctg ctgcggtgcc cgctgaggcc atgccggggc 480
 cccggcgccc cgctggctcc cgctgcgcgc tgcctctgct cctgctgctg ccgcccgtgc 540
 tgctgctgct ccggggcagc cacgcgggca acctgacggt agccgtggta ctgccgctgg 600
 ccaatacctc gtacccttgg tcgtgggcgc gcgtgggacc cgcctggag ctggccctgg 660
 cccaggtgaa ggcgcgcccc gacttgctgc cgggctggac ggtccgcacg gtgctgggca 720
 gcagcgaaaa cgcgctgggc gtctgctccg acaccgcagc gccctggcc gcggtggacc 780
 tcaagtggga gcacaacccc gctgtgttcc tgggccccgg ctgcgtgtac gccgcgccc 840
 cagtggggcg cttcaccccg cactggcggg tcccgtgct gaccgccggc gcccggcgcg 900
 tgggcttcgg tgtcaaggac gagtatgcgc tgaccaccg cgcggggccc agctacgcca 960
 agctggggga cttcgtggcg gcgctgcacc gacggctggg ctgggagcgc caagcgtca 1020
 tgctctacgc ctaccggccg ggtgacgaag agcactgott cttcctcgtg gaggggctgt 1080
 tcatgcgggt ccgcgaccgc ctcaatatta cggtggacca cctggagttc gccgaggacg 1140
 acctcagcca ctacaccagg ctgctgcgga ccatgccgcg caaaggccga gttatctaca 1200
 tctgcagctc ccctgatgcc ttcagaacct tcatgctcct ggccctggaa gctggcttgt 1260
 gtggggagga ctacgttttc ttccacctgg atatctttgg gcaaagcctg caaggtggac 1320

agggccctgc tccccgcagg ccctgggaga gaggggatgg gcaggatgtc agtgcccgcc 1380
aggcctttca ggctgccaaa atcattacat ataaagaccc agataatccc gagtacttgg 1440
aattcctgaa gcagttaaaa cacctggcct atgagcagtt caacttcacc atggaggatg 1500
tcctgggtgaa caccatccca gcatccttcc acgacgggct cctgctctat atccaggcag 1560
tgacggagac tctggcacat gggggaactg ttactgatgg ggagaacatc actcagcgga 1620
tgtggaaccg aagctttcaa ggtgtgacag gatacctgaa aattgatagc agtggcgatc 1680
gggaaacaga cttctccctc tgggatatgg atcccagaaa tggcgccttc agggttgtac 1740
tgaactacaa tgggacttcc caagagctgg tggctgtgtc ggggcgcaaa ctgaactggc 1800
ccctggggta ccctcctcct gacatcccca aatgtggctt tgacaacgaa gaccagcat 1860
gcaaccaaga tcacctttcc accctggagg tgctggcttt ggtgggcagc ctctccttgc 1920
tcggcattct gattgtctcc ttcttcatat acaggaagat gcagctggag aaggaaactgg 1980
cctcgagct gtggcgggtg cgctgggagg acgttgagcc cagtagcctt gagaggcacc 2040
tgcgagtgatg aggcagccgg ctgacctga gcgggagagg ctccaattac ggctccctgc 2100
taaccacaga gggccagttc caagtcttg ccaagacagc atattataag ggcaacctcg 2160
tggctgtgaa acgtgtgaac cgtaaagcga ttgagctgac acgaaaagtc ctgtttgaac 2220
tgaagcatat gcgggatgtg cagaatgaac acctgaccag gtttgtggga gcctgcaccg 2280
accccccaa tatctgcac ctcacagagt actgtccccg tgggagcctg caggacattc 2340
tgagaaatga gagcatcacc ctggactgga tgttccggta ctactcacc aatgacatcg 2400
tcaagggcat gctgtttcta cacaatgggg ctatctgttc ccatgggaac ctcaagtcac 2460
ccaactgcgt ggtagatggg cgctttgtgc tcaagatcac cgactatggg ctggagagct 2520
tcagggacct ggaccagag caaggacaca ccgtttatgc caaaaagctg tggacggccc 2580
ctgagctcct gcgaatggct tcacccctg tcgggggctc ccaggctggg gacgtataca 2640
gctttgggat catccttcag gagattgcc tgaggagtgg ggtcttcac gtggaagggt 2700
tggaacctgag ccccaaagag atcatcgagc gggtgactcg gggtgagcag ccccccctcc 2760
ggccctccct ggccctgcag agtcaoctgg aggagtggg gctgctcatg cagcggtgct 2820
gggctgagga cccacaggag aggccaccat tccagcagat ccgctgacg ttgcgcaaat 2880
ttaacagga gaacagcagc aacatcctgg acaaoctgct gtcccgcag gagcagtacg 2940
cgaacaatct ggaggaactg gtggaggagc ggaccaggc atacctggag gagaagcgca 3000
aggctgaggc cctgcctac cagatcctgc ctactcagt ggctgagcag ctgaagcgtg 3060
gggagacggg gcaggccgaa gcctttgaca gtgttaccat ctacttcagt gacattgtgg 3120

gtttcacagc gctgtcggcg gagagcacgc ccatgcaggt ggtgaccctg ctcaatgacc 3180
 tgtacacttg cttt gatgct gtcatagaca actttgatgt gtacaagggt gagacaattg 3240
 gcgatgccta catgggtggtg tcagggctcc ctgtgcggaa cgggcggcta cacgcctgcg 3300
 aggtagcccg catggccctg gcactgctgg atgctgtgcg ctccctccga atccgccacc 3360
 ggccccagga gcagctgogc ttgcgcattg gcatccacac aggacctgtg tgtgctggag 3420
 tgggtgggact gaagatgccc cgttactgtc tctttgggga tacagtcaac acagcctcaa 3480
 gaatggagtc taatggggaa gccctgaaga tccacttgct ttctgagacc aaggctgtcc 3540
 tggaggagtt tgggtggttc gagctggagc ttcgagggga tgtagaaatg aagggcaaag 3600
 gcaaggttcg gacctactgg ctccctgggg agagggggag tagcaccoga ggctgacctg 3660
 cctcctctcc tatccctcca cacctccct accctgtgcc agaagcaaca gaggtgccag 3720
 gcctcagcct caccacagc agcccatcg ccaaaggatg gaagtaattt gaatagctca 3780
 ggtgtgctta cccagtgaa gacaccagat aggacctctg agaggggact ggcatggggg 3840
 gatctcagag cttacaggct gagccaagcc cacggccatg cacagggaca ctcacacagg 3900
 cacacgcacc tgctctccac ctggactcag gccgggctgg gctgtggatt cctgatcccc 3960
 tcccccccc atgctctcct ccctcagcct tgctaccctg tgacttactg ggaggagaaa 4020
 gagtcacctg aaggggaaca tgaaaagaga ctaggtgaag agagggcagg ggagcccaca 4080
 tctggggctg gccacaata cctgctcccc cgacccctc caccagcag tagacacagt 4140
 gcacagggga gaagaggggt ggcgagaag ggttgggggc ctgtatgcct tgcttctacc 4200
 atgagcagag acaattaa tctttattcc aaaaaaaaaa aaaaaa 4246

<210> 20

<211> 541

<212> PRT

<213> Homo sapiens

<400> 20

Met Pro Ser Leu Leu Val Leu Thr Phe Ser Pro Cys Val Leu Leu Gly
 1 5 10 15

Trp Ala Leu Leu Ala Gly Gly Thr Gly Gly Gly Gly Val Gly Gly Gly
 20 25 30

Gly Gly Gly Ala Gly Ile Gly Gly Gly Arg Gln Glu Arg Glu Ala Leu
 35 40 45

Pro Pro Gln Lys Ile Glu Val Leu Val Leu Leu Pro Gln Asp Asp Ser
50 55 60

Tyr Leu Phe Ser Leu Thr Arg Val Arg Pro Ala Ile Glu Tyr Ala Leu
65 70 75 80

Arg Ser Val Glu Gly Asn Gly Thr Gly Arg Arg Leu Leu Pro Pro Gly
85 90 95

Thr Arg Phe Gln Val Ala Tyr Glu Asp Ser Asp Cys Gly Asn Arg Ala
100 105 110

Leu Phe Ser Leu Val Asp Arg Val Ala Ala Ala Arg Gly Ala Lys Pro
115 120 125

Asp Leu Ile Leu Gly Pro Val Cys Glu Tyr Ala Ala Ala Pro Val Ala
130 135 140

Arg Leu Ala Ser His Trp Asp Leu Pro Met Leu Ser Ala Gly Ala Leu
145 150 155 160

Ala Ala Gly Phe Gln His Lys Asp Ser Glu Tyr Ser His Leu Thr Arg
165 170 175

Val Ala Pro Ala Tyr Ala Lys Met Gly Glu Met Met Leu Ala Leu Phe
180 185 190

Arg His His His Trp Ser Arg Ala Ala Leu Val Tyr Ser Asp Asp Lys
195 200 205

Leu Glu Arg Asn Cys Tyr Phe Thr Leu Glu Gly Val His Glu Val Phe
210 215 220

Gln Glu Glu Gly Leu His Thr Ser Ile Tyr Ser Phe Asp Glu Thr Lys
225 230 235 240

Asp Leu Asp Leu Glu Asp Ile Val Arg Asn Ile Gln Ala Ser Glu Arg
245 250 255

Val Val Ile Met Cys Ala Ser Ser Asp Thr Ile Arg Ser Ile Met Leu
260 265 270

Val Ala His Arg His Gly Met Thr Ser Gly Asp Tyr Ala Phe Phe Asn
275 280 285

Ile Glu Leu Phe Asn Ser Ser Ser Tyr Gly Asp Gly Ser Trp Lys Arg
 290 295 300

Gly Asp Lys His Asp Phe Glu Ala Lys Gln Ala Tyr Ser Ser Leu Gln
 305 310 315 320

Thr Val Thr Leu Leu Arg Thr Val Lys Pro Glu Phe Glu Lys Phe Ser
 325 330 335

Met Glu Val Lys Ser Ser Val Glu Lys Gln Gly Leu Asn Met Glu Asp
 340 345 350

Tyr Val Asn Met Phe Val Glu Gly Phe His Asp Ala Ile Leu Leu Tyr
 355 360 365

Val Leu Ala Leu His Glu Val Leu Arg Ala Gly Tyr Ser Lys Lys Asp
 370 375 380

Gly Gly Lys Ile Ile Gln Gln Thr Trp Asn Arg Thr Phe Glu Gly Ile
 385 390 395 400

Ala Gly Gln Val Ser Ile Asp Ala Asn Gly Asp Arg Tyr Gly Asp Phe
 405 410 415

Ser Val Ile Ala Met Thr Asp Val Glu Ala Gly Thr Gln Glu Val Ile
 420 425 430

Gly Asp Tyr Phe Gly Lys Glu Gly Arg Phe Glu Met Arg Pro Asn Val
 435 440 445

Lys Tyr Pro Trp Gly Pro Leu Lys Leu Arg Ile Asp Glu Asn Arg Ile
 450 455 460

Val Glu His Thr Asn Ser Ser Pro Cys Lys Ser Ser Gly Gly Leu Glu
 465 470 475 480

Glu Ser Ala Val Thr Gly Ile Val Val Gly Ala Leu Leu Gly Ala Gly
 485 490 495

Leu Leu Met Ala Phe Tyr Phe Phe Arg Lys Lys Tyr Arg Ile Thr Ile
 500 505 510

Glu Arg Arg Thr Gln Gln Glu Glu Ser Asn Leu Gly Lys His Arg Glu

515

520

525

Leu Arg Glu Asp Ser Ile Arg Ser His Phe Ser Val Ala
530 535 540

<210> 21
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> siRNA specific for NPR-A

<400> 21
tattacggtg gaccacctgt tcaagagaca ggtgggccac cgtaatattt ttt 53

<210> 22
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> siRNA specific for NPR-A

<400> 22
agaattccag aaacgcagct tcaagagagc tgcgtttctg gaattctttt ttt 53

THIS PAGE BLANK (USPTO)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)